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# Waterborne Transmission of Giardiasis Proceedings of a Symposium Held at Cincinnati, Ohio on September 18-20, 1978

(U.S.) Health Effects Research Lab, Cincinnati, OH

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# Waterborne Transmission of Giardiasis

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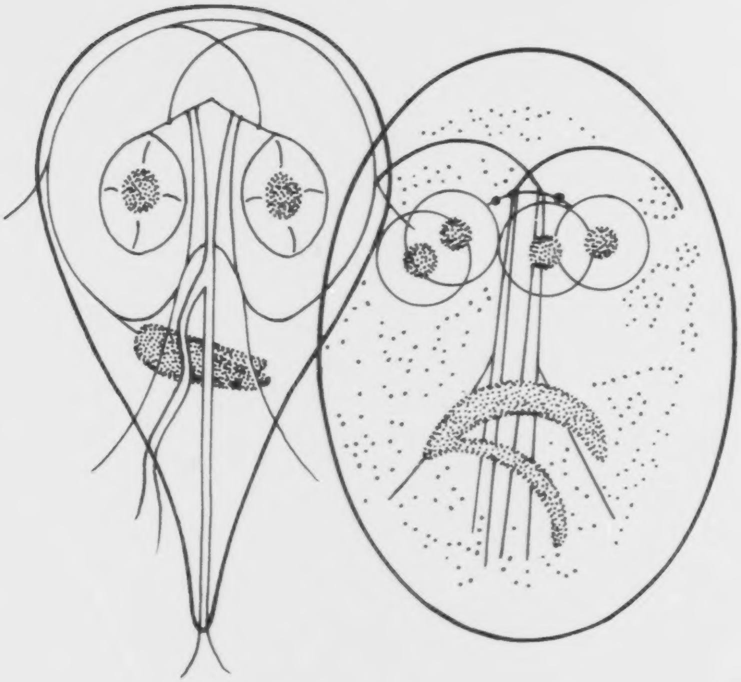

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The Health Effects Research Laboratory and the Municipal Environmental Research Laboratory of the U.S. Environmental Protection Agency sponsored a National Symposium on Waterborne Transmission of Giardiasis in Cincinnati on September 18-20, 1978.

The symposium brought together scientists, engineers, and federal, state, and local public health officials for the purpose of determining the state-of-knowledge regarding the etiologic agent and the disease as they relate to water supplies. Sessions on the organism, the disease, epidemiology, detection methodology, water treatment technology, and research needs were held.

The proceedings consist of 19 invited manuscripts, 6 abstracts of contributed papers and transcripts of discussions following each presentation.

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June 1979

# WATERBORNE TRANSMISSION OF GIARDIASIS

## Proceedings of a Symposium

September 18-20, 1978

Sponsored by the
Health Effects Research Laboratory
and the
Municipal Environmental Research Laboratory

*Edited By*
**W. Jakubowski and J. C. Hoff**

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH CENTER
CINCINNATI, OHIO 45268

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# FOREWORD

The U. S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This report represents an attempt to determine the current state-of-knowledge on the transmission of a pathogenic protozoan agent through drinking water and to define future research needs. With a better understanding of the health effects, measures can be developed to reduce exposure and to provide adequate treatment in order to eliminate this organism as a significant cause of waterborne disease.

R. John Garner, Director
Health Effects Research
Laboratory

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

# PREFACE

The occurrence of recent community-wide outbreaks of giardiasis associated with drinking water supplies has resulted in the recognition that basic information on the etiologic agent and mode of transmission is lacking. The U. S. Environmental Protection Agency has a mandate under the Safe Drinking Water Act of 1974 to identify contaminants that may cause adverse health effects and to either promulgate maximum contaminant levels for drinking water or specify treatment requirements. In order to determine what measures are required to protect the public health, reliable data are needed on the occurrence, epidemiology, and adequacy of available treatment technology. The purpose of this Symposium was to bring together scientists, public health administrators, and water suppliers to discuss various aspects of giardiasis and to provide in a single publication a comprehensive summary of current knowledge.

The Proceedings are organized into seven main sections corresponding to the format of the Symposium and addressing the organism, the disease, epidemiology, detection methods, other recent research, treatment technology, and research needs. One section consists of abstracts of papers presented at an evening session (Session VI - Other Recent Research) and represents relevant research brought to the attention of the Symposium coordinators after the program had been finalized.

In many cases, the Proceedings papers are more comprehensive than the Symposium presentations because of the desire to provide thorough coverage of a given topic. The section on epidemiology contains a paper not presented at the Symposium. As a result of a suggestion made at the Symposium, the coordinators sought and obtained consent from Dr. Lyle Veazie to incorporate in the Proceedings an unpublished manuscript on a suspected outbreak of giardiasis affecting an estimated 50,000 persons in Portland, Oregon in 1954-55. Information concerning this event had previously been reported only in the form of a letter to the New England Journal of Medicine (281:853, Oct. 1969).

Edited discussions are included with papers and an attempt has been made to identify each questioner where possible. A list of registrants is presented, thus enabling the reader to contact any speaker for further information.

Walter Jakubowski
John C. Hoff
*Editors*

# ABSTRACT

The Health Effects Research Laboratory and the Municipal Environmental Research Laboratory of the U. S. Environmental Protection Agency sponsored a National Symposium on Waterborne Transmission of Giardiasis in Cincinnati on September 18-20, 1978.

The Symposium brought together scientists, engineers, and federal, state, and local public health officials for the purpose of determining the state-of-knowledge regarding the etiologic agent and the disease as they relate to water supplies. Sessions on the organism, the disease, epidemiology, detection methodology, water treatment technology, and research needs were held.

The Proceedings consist of 19 invited manuscripts, 6 abstracts of contributed papers and transcripts of discussions following each presentation. A panel discussion on research needs is included.

# ACKNOWLEDGMENTS

The assistance of the many individuals who contributed to the success of this Symposium and the timely completion of the Proceedings is gratefully acknowledged. Special appreciation is due to the speakers, for the quality of their presentations and promptness in submitting their papers, the session chairmen and the many participants who contributed to the discussions.

We also wish to acknowledge the assistance of the Office of Support Services and in particular the efforts of Ms. Kathy Burleigh, of that Office, in providing the many administrative services in connection with arranging the Symposium. We also are indebted to Dixie White, Saundra Underwood and Shirley Jakubowski for assisting with registration, and to Steve Waltrip and Judy Barnick who helped in many ways to make the Symposium a success.

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## GORDON G. ROBECK

*COORDINATOR OF DRINKING WATER RESEARCH*
*U. S. ENVIRONMENTAL PROTECTION AGENCY*

As Mr. F. Mayo indicated in his welcoming speech, I have a dual role in the area of drinking water research: one is to direct the treatment activities within the Municipal Environmental Research Laboratory, and the second is to try to help some of our planners in Washington, D.C. at headquarters to select priorities and thus allocate available resources.

The administrator of the U. S. EPA Office of Research and Development, Dr. Stephen J. Gage, has been trying to integrate the various research needs through a Research Committee. There are many subunits in his office that he is experimenting with now to see how well this integrating process will work. He has formed five Research Committees and named managers for each of these to try to elicit the needs of the cities, regions and states, and to see what the operating programs in Washington need concerning existing legislative mandates. It is in the capacity of a Research Committee Manager that I speak to you today.

As you know, EPA is a regulatory agency, but we are delighted to be able to spend a certain amount of the agency's resources on research, designing our studies to provide a basis for intelligent regulatory action.

As you have heard from our two welcoming speakers, we are here today because we have had an upsurge in the occurrence of giardiasis, particularly in certain areas of the United States. It is somewhat puzzling why it is regionalized, and ironically, in those areas that we frequently call protected watersheds. It is the usually relatively good mountain water that seems to be contributing to the outbreaks. Although better reporting of disease may be a factor, we became particularly apprehensive when we noticed that even where we had reasonably good treatment systems in place we still had outbreaks. As an engineer, that concerned me and made me realize that it was time for a better understanding of several scientific and technical questions, as well as for a better enforcement and training program. However, we had no specific goal or standard for enforcement, so we realized that we had to do more research in order to advise the policy makers and regulators.

For instance, is a combination of the current turbidity limit of one to five and the coliform limit of a monthly average of one adequate to protect the consumer against giardiasis? We have had some outbreaks where both limits have been met, so we are not totally convinced that we have an adequate safety factor built into the microbiological and physical limits.

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EPA's Office of Drinking Water has been contemplating the use of a section in the Safe Drinking Water Act that allows setting a treatment requirement when it is difficult to monitor for a specific contaminant. That option, of course, may involve requiring coagulation and filtration, followed by disinfection, particularly for surface waters. I hope this symposium will help guide us to some sensible conclusion with regard to the needs for future research to support necessary Agency action.

I have been asked to set the tone for this symposium, and to do so I must of course briefly review what we know from the literature that is currently available and what we know also from recent studies that many of you have participated in, and lastly, where we are going in the Agency.

What we know about the organism is that it was reported in the scientific literature as a known entity almost three hundred years ago. It had long been considered non-pathogenic because many people shed the organism and did not seem to be ill. Now, most public health people consider it as a disease to be reckoned with, and we certainly think we know in general terms what the causative agent is and perhaps how to control the disease through treatment as far as water being the medium for transmission.

As most of you know, it has two stages in its life cycle, one the reproductive trophozoite, and the other the cyst stage. It is as a cyst, of course, that it is usually shed by most people. The populations of cysts from different hosts, and even from the same host, actually exhibit variations in size and shape, and we do know that there is a difference in the internal structure of some of these. However, we do not know the number of species of these organisms that exist, nor the host range of the species. Other mysteries are the mechanisms that trigger excystment and encystment. Through the use of the electron microscope, serologic studies and animal infection studies, we may be able to shed some light on further characterizing this organism and determining the relationship of possible host reservoirs to waterborne transmission.

Now, what do we know about the disease itself? The infection is manifested in a variety of ways ranging from asymptomatic to acute disease with severe diarrhea, weight loss, and sometimes retarded growth in children. The occurrence of the disease as well as the efficacy of treatment may depend upon inherent characteristics of the host including immunologic status and the underlying disposition of the individual, or, on the other side of the equation, upon the virulence of the organism itself. The infectious dose appears to be quite low and a large number of cysts can be shed from infected individuals. The serologic response may depend upon the severity of the infection and possible adaptive mechanisms in the organism itself.

We need better methods for diagnosis in order to more accurately assess the true incidence of infection and disease. Also, there are only two drugs of choice for treatment in this country now. One has been shown to be carcinogenic in mice and rats and the other may result in side effects more severe than the infection itself. The possibility that a *Giardia* infection may predispose individuals toward ulcers and gallbladder disease or other conditions should be explored.

The other major area that we want to discuss in this Symposium is the epidemiology of the disease. Here we have studied and observed worldwide that giardiasis is endemic in some places and epidemic in others. The underlying attitude for many years was that it might be a tropical disease, but this has been somewhat negated by evidence that there have been infections and outbreaks in the Yukon, in Russia and in the northern mountainous areas of the United States. These places obviously are not tropical.

We had gradually accumulated the impression that giardiasis was a disease of hikers, campers, hunters, skiers and fishermen who drink untreated water from mountain streams, but in truth we have had more than 20 outbreaks that have been documented in the United States alone since the outbreak in Aspen, Colorado in 1965. Between 1966 and 1974, we have had 14 outbreaks affecting about 400 people, and they generally have occurred in small municipal and semi-public systems in recreational areas. However, in the last four years there have been community-wide outbreaks associated with municipal water supplies resulting in over 6300 cases.

The epidemiologists also tell us that the organism infects a wide variety of domestic and wild animals that may act as reservoirs of infection and certainly sources of contamination of water supplies. Beavers, for instance, were implicated in the Camas, Washington outbreak of 1976. Therefore, we need to determine the sources of contamination of our water supplies, understand the seasonal occurrence in domestic and wild animals and human populations, and also determine the significance of water supplies for transmitting and maintaining the infection throughout the population.

How well can we quantify and identify this organism? Actually, there is no good cultural method for detecting and enumerating the organism, and just to give some perspective as to where we are in our inability, just imagine gathering data on coliform levels in water by doing microscopic counts of the organisms. That is basically where we are with *Giardia*. During the Rome, New York outbreak of 1974-1975, the Center for Disease Control developed a sand filter that they hoped would allow them to concentrate the cysts in large enough numbers to be able to detect them microscopically and to determine their infectivity. This method was successful but the size of this unit, unfortunately, made it very difficult to transport and to collect samples in areas a considerable distance away from Atlanta. The Health Effects Research Laboratory here at EPA developed a portable water sampling device that has since been successfully used to detect the cysts in the Camas, Washington; Berlin, New Hampshire; and, Vail, Colorado outbreaks. However, this method remains to be evaluated under a variety of sampling conditions. The microscopic detection of cysts does not supply information about the species of origin or viability, but recent work at the University of Oregon may lead the way to development of a cultural technique and a method for determining viability.

What do we know about controlling the occurrence of the disease, presuming that it can be spread not only by humans contaminating watersheds, but by infected animals, which may be very difficult to control? Outbreaks have occurred, as I have mentioned, in several states, but there was no treatment to speak of or else there had been a breakdown in the existing treatment. The effect of various disinfectants, such as chlorine, or some of the suggested new ones such as ozone, ultraviolet radiation or chlorine dioxide, are not well known. We had been trying to extrapolate from *Entamoeba histolytica* data, and you will be discussing later this week the appropriateness of that laboratory information to the *Giardia* situation. We certainly need to determine the optimum parameters for removal of cysts by flocculation, filtration and by disinfection, particularly in the small systems. Throughout the last year or so we have found through implementation of the Safe Drinking Water Act that most noncompliance situations develop in the small systems. Therefore, we are turning more and more of our resources and research toward addressing that problem.

It seems that many people have reasonsed in the past that we have the technology for controlling not only organisms but certain factors such as turbidity created by clay and other runoff products, as well as certain chemicals, but the newness of the Safe Drinking Water Act, and its application to such small systems, soon taught us that most of the small utilities were simply not aware of the feasibility of using some of the available technology. We must have good training programs, and less expensive systems which are easier to operate than current systems. We also must make sure that there are skilled people commensurate with the sophistication of equipment.

We feel that the Administrator of EPA will find the results of this conference a great aid to his decision making process. If we can develop the appropriate information, we may be able to show that treatment of all surface waters by coagulation and filtration is a necessity, and if operated properly, we can minimize the amount of disinfectant used. In the past, superchlorination and dechlorination have been used as a control measure for *Giardia lamblia*, but this could result in creating trihalomethanes, and this then means that one public health practice is resulting in another public health problem.

I want to once again thank you for attending and I look forward to hearing your presentations and the ensuing discussions.

# SESSION I - THE ORGANISM

*Chairman - George J. Jackson,*
*Food and Drug Administration, Washington, D.C.*

**_Giardia lamblia:_ Classification,**
**Structure, Identification**
**N. D. Levine**

**Ultrastructural Aspects of _Giardia_**
**H. G. Sheffield**

**Surface Morphology of _Giardia_ Cysts**
**Recovered from a Variety of Hosts**
**A. S. Tombes**

# *Giardia Lamblia:* Classification, Structure, Identification

**Norman D. Levine**

*College of Veterinary Medicine and Agricultural Experiment Station, Univ. of Illinois, Urbana, Illinois*

## ABSTRACT

*Giardia lamblia* Kofoid and Christiansen, 1915 is a flagellated protozoon belonging to the class Zoomastigophorasida, order Diplomonadorida, family Hexamitidae. It is found in the small intestine of man and other mammals. Its trophozoites are 9 to 21 µm long, 5 to 15 µm wide and 2 to 4 µm thick. They have a piriform body with a broaely rounded anterior end, 2 nuclei, 2 slender median rods, 8 flagella in 4 pairs, a pair of darkly staining median bodies, and a large, ventral sucking disk. Its cysts are ovoid, 8 to 12 x 7 to 10 µm, with 4 nuclei and remnants of the organelles. Its trophozoites are easily identified in moist scrapings of the intestinal mucosa from their shape and characteristic motion. They can also be identified in fixed and stained smears from their structure. Its cysts can be concentrated by zinc sulfate (but not sugar) flotation, and can be identified from their structure.

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## CLASSIFICATION

*Giardia lamblia* is a protozoon which has generated much interest in recent years. It has been taken as the emblem of the International Congresses of Protozoology, the first of which was held in Prague, Czechoslovakia in 1961 and the fifth in New York City in August, 1977. *Giardia lamblia* has also emerged as a cause of human disease. The classification of the genus would be a simple matter if it were based on modern knowledge alone, but the nomenclature of its species is another matter.

To elucidate; Leeuwenhoek was the first to see *Giardia*—in 1681 in his own stools. The genus itself was named by Joseph Kunstler in 1882; Raphaël Anatole Émile Blanchard called it *Lamblia* in 1888, but he was 6 years too late, and this generic name is a synonym of *Giardia*. Despite this fact, some Europeans still use the name *Lamblia*. Reichenow(1) compromised; he called the frog species *Giardia agilis* and the human one *Lamblia intestinalis*.

Vilem Dusan Fedorovic Lambl found the human organism in 1859 and called it *Cercomonas intestinalis*. In 1879 Giovanni Battista Grassi found *Giardia* in the house mouse and called it *Dimorphus muris*; in 1881 he found it in man and called it *Megastoma entericum*. Unfortunately, he also used this same name for giardias in *Mus, Apodemus* and the cat, and thus compromised it. The species name *intestinalis* was found to be a homonym of the organism that Diesing had called *Cercomonas intestinalis* in 1850, and the species name *enterica* was suppressed as a synonym of *Giardia muris*.

Most Europeans still call the human species *Giardia intestinalis*, ignoring the fact that this species name is a homonym. Corradetti(2) made a good case for calling it *Giardia enterica*. Although not stated by Corradetti(2), the median bodies of the human form are curved bars resembling the claw of a claw-hammer while those of *G. muris* are small and rounded. Since this is the case, *G. enterica* cannot be a synonym of *G. muris*, and the name is available for the human species.

There had been so much confusion about the availability of the specific names, *intestinalis* and *enterica*, that Charles Wardell Stiles established the name *Giardia lamblia* in a letter to Kofoid and Christiansen(3); therefore, this is the name that we use.

Using the light microscope, Filice(4) was unable to find any structural difference between the giardias of the laboratory rat and a number of wild rodents. On reviewing the literature, he discovered that almost no acceptable cross-transmission studies existed between some species. He appears to have concluded that there are only 2 species in mammals, each having a number of races and that these species differ only in their median bodies. *G. muris* occurs in the house mouse, rat and hamster, and has rounded median bodies. *G. duodenalis* (Davaine, 1875) occurs in the rabbit, cottontail, Norway rat (*G. simoni*), golden hamster, guinea pig, man, and presumably ox, dog, cat, etc., and has median bodies shaped like the claws of a claw-hammer. Since *duodenalis* was the first name (barring *intestinalis* and *enterica*) given to a claw-hammer *Giardia*, this was the species name he used. Bemrick(5) agreed with this point of view, and accepted only the names *G. muris* and *G. duodenalis* for the mammalian species.

Many individuals,—myself among them—have used different names for the claw-hammer giardias from different hosts. We speak of *G. lamblia* or *G. intestinalis* or *G. enterica* from man, *G. canis* from the dog, *G. cati* from the cat, *G. bovis* from the ox, *G. duodenalis* from the rabbit, *G. simoni* from the rat and mouse, etc. This practice, which is a matter of convenience, ignores the possibility that they are all the same species and can be transmitted from one host to another. At present it would appear that cross-transmission between various hosts is common. This, of course, is one of the reasons for our present symposium. If this is the case, I suppose that the purists among us will want to call the organism *Giardia enterica* (Grassi, 1881).

Now, forgetting this aspect of the problem, how about the classification of the genus? *Giardia* is a flagellate, and is classified as follows.

Phylum-SARCOMASTIGOPHORA Honigberg & Balamuth, 1963

With a single type of nucleus, vesicular; sexuality, if present, syngamy; with flagella, pseudopodia, or both types of locomotory organelles; typically no spore formation.

Subphylum-MASTIGOPHORA Diesing, 1866

One or more flagella typically present in trophozoites; asexual reproduction basically by longitudinal binary fission; sexual reproduction unknown in many groups.

Class-ZOOMASTIGOPHORASIDA Calkins, 1909

Chromatophores absent; 1 to many flagella; amoeboid forms with or without flagella in some groups; sexuality absent in most groups; predominantly parasitic.

Order-DIPLOMONADORIDA Wenyon, 1926

With 2 karyomastigonts, each with 4 flagella; at least 1 of these flagella is recurrent; with 2 nuclei; without mitochondria or Golgi apparatus; with cysts; free-living or parasitic.

Family-HEXAMITIDAE Kent, 1880

With at least 1 recurrent flagellum per karyomastigont.

This is the family within which *Giardia* occurs, and the only family in the order. It contains 5 genera. Brugerolle(6,7) made an electron microscope study of the group and divided this family into 2 subfamilies, based on whether there are functional cytostomes or not. In the subfamily Hexamitinae Kent, 1880, each recurrent flagellum lies in a hollow tube or depression which forms a functional cytostome; whereas in the subfamily Octomitinae the recurrent flagella lie in the cytoplasm itself and there are no cytostomes.

In the subfamily Hexamitinae there are 3 genera: *Trepomonas* Dujardin, 1838, with 2 flagella directed forward and 6 directed backward and lying in 2 lateral fossae or cytostomes; *Hexamita* Dujardin, 1838, with a broad body, subspherical nuclei, and 6 flagella directed forward and arising external to the nuclei, and with the 2 recurrent flagella in cytostomal tubes; and *Spironucleus* Lavier, 1936, like *Hexamita* but with an elongate body and elongate, S-shaped nuclei.

In the subfamily Octomitinae there are 2 genera: *Octomitus* von Prowazek, 1904, with 6 anterior and 2 recurrent flagella, without a sucking disk, and *Giardia* Kunstler, 1882, with the same number of flagella as *Octomitus*, but with a sucking disk.

The evolutionary relationships of these genera are shown in Fig. 1, taken from Brugerolle(6,7). Those relationships are different from illustrations based on light microscope studies which are found in textbooks. Note that Brugerolle considered that *Trepomonas* arose from *Enteromonas* by longitudinal doubling. He was so sure of this that he put *Enteromonas* in the Diplomonadorida even though it is not doubled. Levine(8), called it Incertae Sedis because he didn't know where to put it. Brugerolle said that the 5 genera—*Trepomonas, Hexamita, Spironucleus, Octomitus* and *Giardia*—form a linear series, each one arising from the one below it. The parasitic genera arose from the free-living ones, which are shown to the left of the dashed lines.

All species of *Trepomonas* are free-living, some species of *Hexamita* are free-living and some are parasitic and all species of *Spironucleus, Octomitus* and *Giardia* are parasitic. The diagram (Fig. 1) indicates that *Enteromonas* is free-living but this is not true. It has been found in the intestine of a number of mammals, including man, pigs, rats and rabbits. The forms that

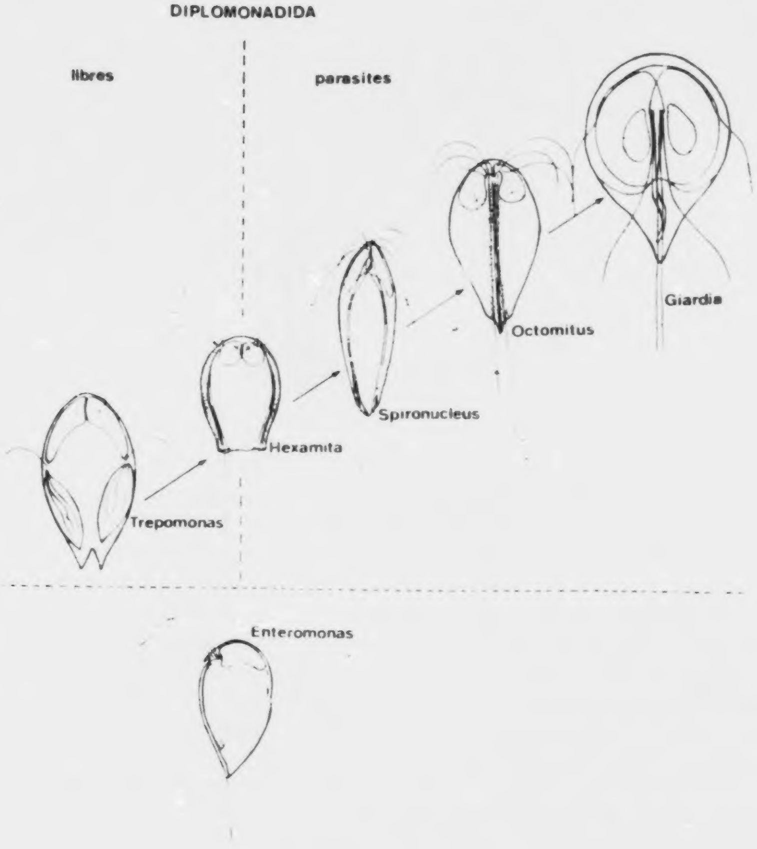
DIPLOMONADIDA
libres
parasites
Giardia
Octomitus
Spironucleus
Hexamita
Trepomonas
Enteromonas

FIG. 1. ***Evolutionary relationships of genera in the family Hexamitidae(6).***

Brugerolle studied came from the intestines of a salamander and the domestic rabbit.

I think that I have confused you enough. Fig. 2 is a drawing of the organism under discussion today—*Giardia lamblia* or whatever you want to call it. The drawing shows both the trophozoite and the cyst.

## STRUCTURE

The fine structure of *Giardia* as seen through the electron microscope will be discussed by others (see Sheffield, these Proceedings). However, I think that I might review for you what you would see with the light microscope.

The body is rather pear-shaped, with a broad anterior end which comes to a blunt point posteriorly. It is bilaterally symmetrical. There is a large sucker, or sucking disk, on the ventral side; the dorsal side is convex. There are 2 anterior, vesicular nuclei, and 2 recurrent flagella running back

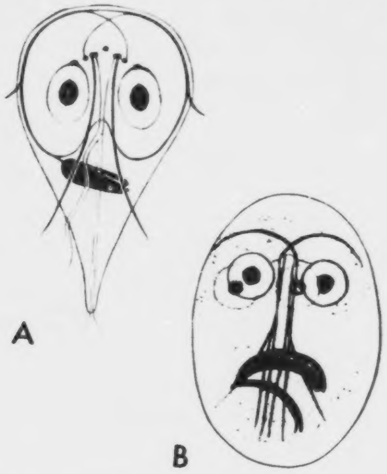

FIG. 2. *Trophozoite (A) and cyst (B) of* **Giardia lamblia**. *A after Filice (4); B from Becker & Frye(10).*

through the middle of the body. These have been referred to as median rods or axostyles; however, they really are not axostyles. There are 4 pairs of flagella. (They have been cut off in the drawing—and are actually much longer than shown here). There are 2 median bodies composed of bundles of microtubules arranged either irregularly or sometimes united in ribbons. They disappear in the course of division and perhaps have something to do with formation of the new sucking disk.

The cysts have 4 nuclei when mature, plus a variable number of fibrillar remnants of the trophozoite organelles.

## IDENTIFICATION

*Giardia* can be identified either while alive—at least the trophozoites can be—or after fixation and staining. To identify the live trophozoites, make a scraping of the duodenum, mix with a little physiological NaCl solution, and examine under the oil immersion objective of the microscope. They are 9 to 21 μm long, 5 to 15 μm wide and 2 to 4 μm thick. They can be readily recognized from their appearance and from their sort of flopping motion. The cysts are ovoid to ellipsoidal, 8 to 12 x 7 to 10 μm.

Trophozoites and cysts can also be identified after fixation and staining. For fixation Schaudinn's fixative (2 parts saturated mercuric chloride solution and 1 part 95% alcohol, plus 5% acetic acid added immediately before use) is recommended. For staining, Heidenhain's hematoxylin is best. Directions for its preparation and use are given in Levine(8).

Cysts can be concentrated by flotation with a solution of greater density. Such flotation solutions are used routinely for fecal examination, but can also be adapted to use with water samples. They are worthless for trophozoites, however, and suitable only for cysts, which may or may not be present in water.

Not all flotation solutions are suitable. Sugar solutions are unsatisfactory, and I suspect that NaCl solutions are also. The cyst wall is actually quite delicate, and I have watched *Giardia* cysts that have been concentrated by sugar flotation shrivel and become unrecognizable in a matter of minutes. I recommend zinc sulfate flotation. Hampel(9) developed a modification of this technic, layering 1% potassium alum solution on the surface of the zinc sulfate solution, recentrifuging, and staining with 1% eosin solution.

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## Discussion

W. JAKUBOWSKI: You mentioned median body differences in the *muris* and *lamblia* trophozoites. Do these differences carry over into the cyst stage?

N. LEVINE: As far as I know, they do.

W. JAKUBOWSKI: Then you believe it would be possible to differentiate cysts of *muris* and other species by the appearance of the median bodies?

N. LEVINE: If you get them early enough, but later on the median bodies will degenerate and you will not find them at all.

W. JAKUBOWSKI: You also mentioned the maturation period required for cysts and the presence of four nuclei in mature cysts. Can you say anything about how long this maturation period might take and what changes go on?

N. LEVINE: I cannot tell you. It takes a while.

B. MYERS: In speciation, how do you distinguish rodent *Giardia* from *Giardia* in beaver, dog and so on?

N. LEVINE: If they have claw hammer type median bodies, the only way that I can tell them apart is by measurements, and that is not very good. I have looked at *Giardia* in the dog, various ruminants and humans, and I cannot tell them apart. They all look the same except for *muris*. But in mice and rats, also, you have not only *muris* but *simoni* with claw hammer median bodies.

SPEAKER: Could you give a little more detail on those flotation techniques? What was the problem with the sugar flotation?

N. LEVINE: The sugar must penetrate and shrink the cysts. They shrivel up and you cannot recognize them at all after a few minutes. That is why I think you have to use zinc sulfate for flotation.

The technique itself is rather simple. Use sucrose in water, and a little carbolic acid (phenol) as a preservative. Then use half of that and half of the fecal suspension in water or physiological salt solution, and either centrifuge it for five minutes or let it stand for about a half hour. I use an ordinary test tube that has been ground off perfectly flat on top and place a round cover slip on it, and then pick off the cover slip and place it on a slide for examination.

D. STEVENS: This is a minor detail, but we have used a sucrose flotation technique, and we watched cysts immediately after removing them from the interface and placing them in physiological saline, and we have not noted the same problem in morphology, but this is with a rodent strain. As long as you pull them right off, there does not seem to be a problem.

N. LEVINE: Yes, it ought to work all right. I never tried that. I have just used the standard flotation technique.

G. HEALY: I remember reading a review paper in French on the species of *Giardia*, (Ansari, M.A.R., *Pakistan J. Health*, 4:131, 174, 1954-55), and the thing that bothered me was that he had some pretty good evidence for tremendous differences in cyst size of various species. I wonder what your thought is about this in terms of having differentiation based on the claw hammer or the smooth type of median bodies.

N. LEVINE: I am suspicious of anything that depends entirely on size because it is possible that the size may be just a race character or be influenced by the host, so I just do not know.

# The Ultrastructural Aspects of *Giardia*

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## ABSTRACT

Transmission and scanning electron microscopy has provided new information on the structure and possible function of the various organelles in the trophozoite and cyst of *Giardia* spp. The adhesive disk consists of numerous parallel microtubules lying adjacent to the ventral plasma membrane. The microtubules are attached to each other by filamentous bridges and each has a ribbon-like structure extending dorsally from it. Contraction of the adhesive disk, attachment by the ventrolateral flange, or negative pressure produced by flagellar motion under the disk has been proposed as a means of attachment of the organism to the host intestine. Median bodies have been identified as groups of microtubules. Numerous vacuoles along the periphery of the organism have been proposed as feeding organelles or secretory vesicles involved in encystment. These various organelles are also seen in the cyst but are often disorganized. In addition, the cyst has a thin fibrous cyst wall to protect it from the environment outside of the host. This paper reviews the ultrastructure of the trophozoite and cyst.

*Giardia lamblia,* which infects man, and various other species of the genus *Giardia* have been of interest to light microscopists for many years. In fact, *G. lamblia* was among the first of the parasitic protozoa to be seen with the microscope. Judging from his detailed description, Leeuwenhoek probably observed the trophozoite of *G. lamblia* while studying his own feces(1). Over the years, the morphological characteristics of *Giardia* have been studied by many workers. The distinct appearance of the trophozoite has amused biology students as well as being used as the emblem for the International Congresses of Protozoology. When electron microscopes became available to protozoologists, Rossi-Espagnet and Piccardo(2) were the first to examine *Giardia*. However, their technique of utilizing whole cells did not yield sufficient resolution to augment existing knowledge derived by light microscopy. In this presentation, the morphology of the trophozoite and the cyst of *Giardia* as determined by transmission and scanning electron microscopy will be reviewed.

The trophozoite, when viewed from the dorsal surface, is pear-shaped with the anterior end broadly rounded and the posterior end pointed. The dorsal surface is convex whereas the ventral surface is somewhat concave. Eight bilaterally paired flagella are present. Their basal bodies arise near the midline at the level of the bilaterally situated nuclei. Two of the flagella emerge anterolaterally, two posterolaterally, two ventrally and two caudally. The distinctive sucking disk occupies most of the anterior ventral surface (Fig. 1).

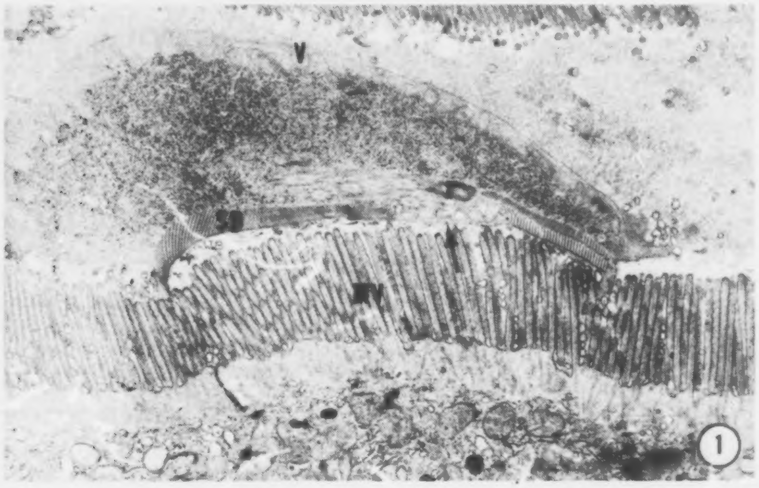

FIG. 1. **Giardia sp.** *in dog small intestine. 10,500X. Trophozoite attached to microvillar border of an epithelial cell. Abbreviations for all figures: AS, axostyle, AX, axoneme; CW, cyst wall; L, lacuna; MB, median body; MV, microvilli; PM, plasma membrane; S, endosymbiont; SD, striated disk; SL, striated layer; V, vacuole; VF, ventrolateral flange.*

The fine structure of the sucking disk was first demonstrated by Cheissin(3) who studied *Lamblia (Giardia) duodenalis* obtained from rabbits and grown in culture. He described the sucking disk as having two lobes which were fused between the nuclei. The pellicle of the sucking disk had a characteristic striation due to alternating light and dense lines. These lines, or "ridges" as he termed them, were interpreted to be infoldings of an inner membrane which was continuous and located just internal to the ventral plasma membrane. The ridges were concentric within the centers of the lobes but were longitudinally arranged at the margins. At the midline, anterior to the nuclei, was an area devoid of the striations which was termed the "naked area". Morecki and Parker(4) studied *Giardia lamblia* obtained from human biopsies. They also found that the sucking disk occupied the ventral portion of the entire cephalic pole, except for the naked area, and a caudal extension of the latter termed the ventral groove. They also described two additional lobes which were situated on either side of the ventral groove. These lobes fused at their caudal ends. The structure of the sucking disk consisted of ridges which were perpendicular to the ventral surface and extended 100 to 200$\mu$m into the cytoplasm. In contrast to the observations of Cheissin(3), Morecki and Parker(4) described the ridges as electron-opaque lines 20-30nm in thickness which were interconnected at their bases by circular profiles. They did not find evidence of striation within the ridges.

The ultrastructure of *G. muris* trophozoites was reported by Friend(5), who described the sucking disk (which he termed "adhesive disk") to consist of a single layer of microtubules each of which had a thin fibrous band extending dorsally from its wall. The microtubules were situated about 200Å from the ventral cell membrane. They were about 250 to 300Å in diameter and were connected to adjacent microtubules by slender filamentous bridges. When cut in cross-section, the fibrous bands, or ribbons, were observed extending dorsally to the endoplasm and had a thickness of 200Å. Longitudinal sections indicated a fine cross-banding at the ribbons.

*Lamblia (Giardia) muris* was also studied by Soloviev and Chentsov(6). Their work corroborated that of Friend(5) and added that the ribbons were approximately 2000Å in height and were spaced about 300Å apart. The thickness of 80Å was somewhat less than that observed by Friend.

Thus far workers agreed, in general, that the sucking disk was composed of perpendicular ribbons attached to microtubules aligned along the ventral plasma membrane. This type of structure does not suggest one of motility but rather one of support. For this reason, Friend(5) chose to rename the structure calling it a "striated disk". If the striated disk serves only a cytoskeletal function, what is the mechanism by which the parasite maintains its association with the microvillar surface of the intestinal cells? One possible answer may lie in the function of the marginal lip of the pellicle. This structure was first observed by Cheissin(3) and later described by Friend(5) as the ventrolateral flange. It is an extension of the cytoplasmic dorsal portion of the parasite to the periphery beyond the edges of the striated disk. The ventrolateral flange consists of a finely striated layer which separates the striated disk from the endoplasmic portion of the cell on its medial side and extends outward forming a thin flange covered by the plasma membrane. The striated portion is composed of alternating light and dark bands. Nearer the periphery the bands are more dense. In random cross-sections, the configuration of the ventrolateral flange varies considerably, both in the same organism and between organisms, indicating it to be a flexible structure (Fig. 2).

Involvement of the ventrolateral flange in attachment of the organism to the host mucosa was suggested by Friend(5). The structure was seen in intimate contact with the microvillar border of the epithelial cells and the inner portion of the flange, when cut tangential to the fine striations, displayed a structure and periodicity similar to that of paramyosin obtained by various extraction methods. Friend did not identify the material in the flange but suggested that if it were a contractile protein it would give the necessary motility to the flange.

In his study of attachment of *G. muris* Holberton(7) questioned the traditional acceptance of the ventral disk (striated disk) as the organelle of attachment. However, his findings supported the old view and he concluded that Friend's(5) hypothesis was incorrect. In a detailed description of the

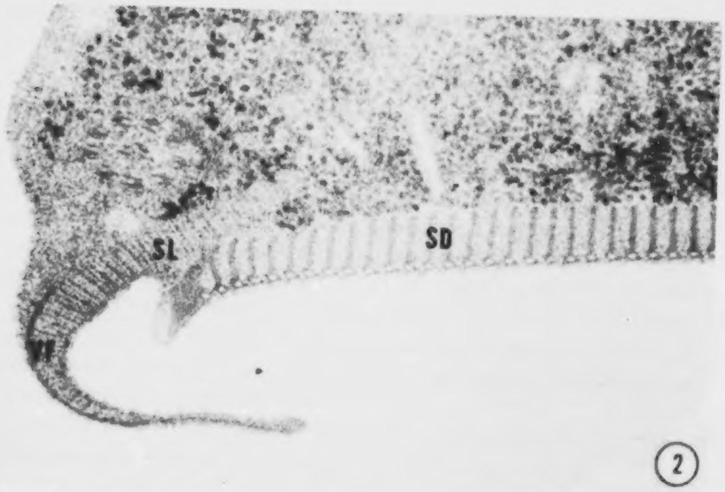

FIG. 2. **Giardia muris.** *74,000X. Section through lateral portion of trophozoite showing the ribbons and microtubules of the striated disk, the ventrolateral flange and the striated layer extending into the flange.*

ventral disk and the association of it with the host mucosa, Holberton pointed out that the incurved edge of the ventral disk penetrates the host enteric surface coat and often becomes situated between the epithelial cell microvilli. The ventrolateral flange was observed to lie against the microvillar surface coat but rarely in contact with the plasma membrane of the cell. The ventral disk was reported to be, with little doubt, the organelle of attachment. Study of the microtubules and their side-arms indicated that a sliding action could take place but the degree of motion would be limited by the large number of cross-bridges between the ribbons of the disk. Further examination of other parameters such as disk diameter, curvature of the ventral surface and inflection of the disk rim did not support the hypothesis that mechanical deformation of the disk was responsible for adhesion. Thus, while disagreeing with Friend on the role of the ventrolateral flange in attachment, Holberton also concluded that the ventral disk was a rigid supportive structure.

Although the electron microscope had provided much new information on *Giardia* structure, it was the light microscope that facilitated further investigation of adhesion of the parasite to the substrate. In the study mentioned above, Holberton(7) observed, by phase microscopy, living trophozoites obtained from mice. In motile cells all flagella seemed to contribute to the motion. However, when the parasite became attached, the anterior, posterolateral and caudal flagella were quiescent whereas the ventral flagella beat with a vigorous, synchronous rhythym. Such beating produced a strong fluid flow through the ventrocaudal groove. This fluid flow was postulated to produce an area of lowered pressure in the region of the ventral disk which resulted in the adhesive force necessary to hold the parasite against the mucosa. This postulate was further supported by the fact that when ventral flagellar motion ceased, the parasites became detached from the substrate. The mathematical basis for such an attachment mechanism was presented in a later study by Holberton(8).

An additional hypothesis for parasite attachment was briefly presented by Mueller, Jones and Brandborg(11). Using scanning electron microscopy in their study of *G. lamblia*, views of both the ventral surface of the parasite and surface views of the intestinal brush border where parasites had been attached, indicated that the striated disk was not a bilaterally symetrical, bilobed disk but an overlapping spiral of parallel lamellae. They suggested that adhesion of the disk was effected by contraction of the microtubules of the spiral causing the edges of the disk to be pulled inward and down between the microvilli of the intestinal cells. Support for their interpretation is found in the work of Brugerolle(12) who, in a detailed study of the striated disk, reported that it was formed by a helicoidal helix of microtubules bearing the ribbon-like structures.

Another structure, seen mainly in *Giardia* trophozoites, that has received attention is the median body. This organelle, readily visible by light microscopy, was first studied by Cheissin(3). He described it as a group of straight or slightly curved double membranes which, when cut in cross-section, displayed circular profiles indicating them to be tubules. He found no connections between the median bodies and other organelles of the parasite and concluded that the median bodies were probably lipoprotein reserves playing a role in parasite energetics. Similar descriptions of the median bodies of *G. lamblia* consisting of groups of randomly arranged microtubules have been reported by Takano and Yardley(9); Morecki and Parker(4); and Brooks et al(10).

Friend(5) studied the median body of *G. muris* in detail. He found a similar random arrangement of microtubules which did not have origin or insertion into other structures. In addition, he noted the presence of ventral extensions from the median body which were composed of 6 to 14 microtubules in regular rows. In comparing the organization, structure, incidence and attachments of the median body with the axostyle of other protozoa, Friend concluded that the two were not analogous. Considering their location in the caudal portion of the organism and the fact that they disappear upon encystment, he suggested that they served a supporting function in the posterior portion of the parasite which did not contain the striated disk.

Near the dorsal surface of all *Giardia* thus far examined, there are a series of vacuoles which, in section, range in shape from circular to oval. They tend to be aligned in a single row separating the granular cytoplasm from the plasma membrane. Similar vacuoles are seen in the naked area of the ventral surface and posterior to the lobes of the striated disk(5). Friend(5) compared the structure and location of the vacuoles with mucocysts of other protozoa and suggested that they might be involved in the encystment process. In

1968, Bockman and Winborn(13) reported the uptake of ferritin by *G. muris*. One hour after injection of ferritin into the intestinal lumen of infected hamsters, all vacuoles contained a high concentration of ferritin. Ferritin was also found attached to the cell membrane. Because of the observed close association of the vacuoles with the cell membrane, Bockman and Winborn(13) suggested that a connection between the vacuole interior and the intestinal lumen might exist. Recent studies (personal communication, Robert L. Owen, VA Hospital, San Francisco, California) indicate that the vacuoles are interconnected and form a system of intercytoplasmic canals (Fig. 3). The peripheral location of the vacuoles, both dorsal and ventral, provides them intimate contact with the contents of the intestinal lumen and the mucosal surface of the epithelial cells. This and the ferritin uptake study(14) suggest that the vacuoles could be involved in food uptake. On the other hand, their similarity to secretory organelles of other protozoa supports their role in encystment. Obviously, more work should be done to elucidate the function of this important organelle.

Although *Giardia* has abundant ribosomes in its cytoplasm, it is lacking other common cytoplasmic organelles. Thus far, no one has identified typical mitochondria, Golgi vesicles nor endoplasmic reticulum, although Owens (personal communication) has seen what appears to be rough endoplasmic reticulum in *G. muris* trophozoites.

Transmission of *Giardia* occurs via the cyst. Trophozoites which are sensitive to environmental changes form cysts in the intestine. These cysts are enclosed by a resistant wall and are able to survive until ingested by the new host. Multiplication of the organism also occurs during the cyst stage. Although the cyst is important in the etiology of giardiasis, little work has been done on its ultrastructure. Soloviev and Chentsov(14) reported the general architecture of the cyst of *Lamblia (Giardia) muris*. They observed a homogenous cyst wall of 0.3 to 0.4μm thickness which was separated from the enclosed parasite. The cytoplasm contained ribosomes, vacuoles, flagellar axonemes and rounded formations surrounded by a double membrane. The rounded formations may represent bacteria as recently observed by Owens (personal communication). Soloviev and Chentsov(14) did not see endoplasmic reticulum, mitochondria, nor Golgi apparatus but did report fragments of the striated disk dispersed in the cytoplasm.

Similar findings were reported from *G. lamblia* by Sheffield and Bjorvatn(15). Cysts of *G. lamblia* isolated from a patient with a heavy infection, were surrounded by a cyst wall 0.3μm in thickness and composed of thin fibrous elements interspersed with fine particles (Fig. 4). The same fine fibrous network was observed in the cyst wall of *G. muris* (Sheffield, unpublished observations). The mechanism of protection of the parasite by the cyst wall could not be ascertained from the electron micrographs but one might speculate that the fibrous network might impede large molecules while the membrane at the inner edge of the fibrous layer might function in controlling the passage of small molecules. In *G. lamblia* a thin cytoplasmic layer was associated with the cyst wall and separated from it by the plasma

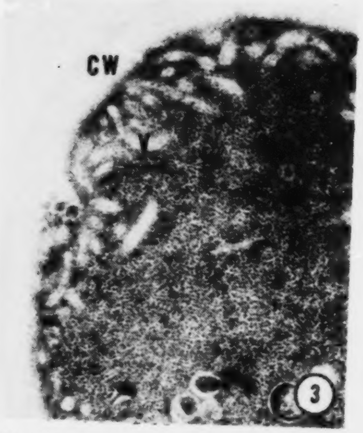

FIG. 3. **Giardia muris** *cyst. 25,750X. Vacuoles along periphery of the organism have the appearance of canaliculi in the cytoplasm.*

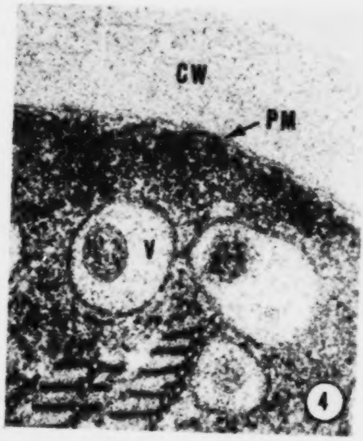

FIG. 4. **Giardia lamblia** *cyst. 52,800X. Cyst wall is composed of fine fibrous material with interspersed small particles. Note plasma membrane of parasite and peripheral vacuoles containing membrane-bound granules. (Sheffield and Bjorvatn. 1977. Courtesy of American Journal of Tropical Medicine and Hygiene).*

membrane. In opportune sections, the direct relationship of the main cytoplasmic mass of the parasite and that underlying the cyst wall was clearly evident (Fig. 5). As reported by Soloviev and Chentsov(14) and in our unpublished observations of the murine giardia, the parasite's central mass appeared to be separate from the thin layer adjacent to the cyst wall. Direct connection has not been seen, although in some areas protrusions from the thin layer extend inward and appear to contact the main body (Fig. 6).

The possibility, suggested above, that the vacuoles around the periphery of the trophozoite are involved in cyst formation is not supported by observations on the structure of the cyst. In both those cysts in which the parasite fills the interior and those in which it has separated from the cyst wall, there are numerous vacuoles close to the plasma membrane. These vacuoles have the same appearance and contain the same flocculent material seen in the vacuoles of the trophozoite. If they functioned as secretory vesicles for cyst wall formation, one would not expect to find them in abundance subsequent to wall formation. Likewise, if they functioned in nutrition of the parasite, one would not expect to find them in the cyst which is apparently a non-feeding stage.

The various organelles have a random arrangement in the cyst cytoplasm. Most noteworthy, and unusual, are the fragments of the striated disk. These structures present various appearances depending upon the angle of sectioning (Fig. 7-9). When cut perpendicular to the microtubule, one sees the circular profile of the microtubule with the ribbon extending from it.

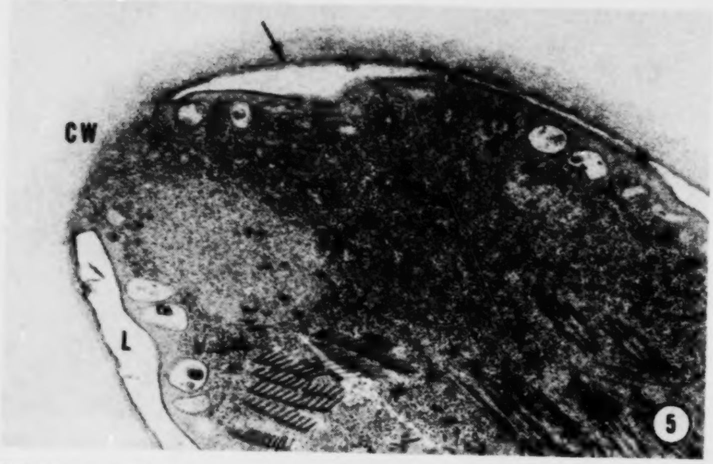

FIG. 5. **Giardia lamblia** *cyst. 20,900X. Main portion of cystoplasm is separated from cyst wall by large lacunae. A thin layer of cystoplasm (arrow) lines the inside of the cyst wall and is connected to the main body.*

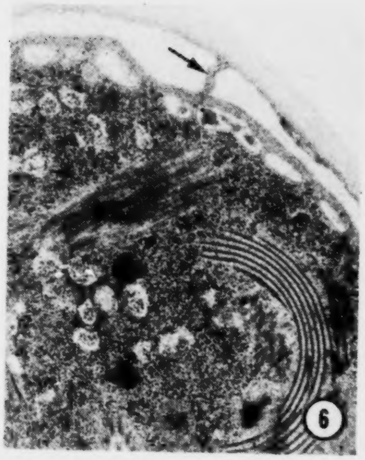

FIG. 6. **Giardia muris** *cyst. 25,000X. Projections from the thin cytoplasmic layer extend inward (arrow) and appear to contact main cytoplasmic body. Note median body microtubules and fragments of the striated disk.*

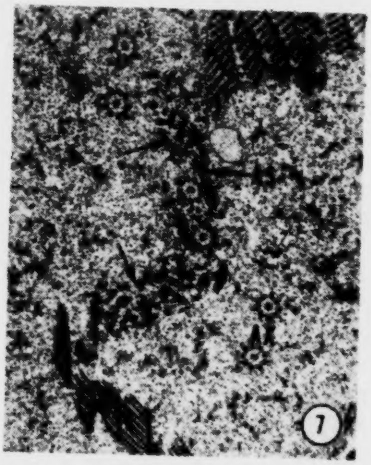

FIG. 7. **Giardia lamblia** *cyst. 22,300X. Section through the axonemes of three pairs of flagella. Two groups of randomly oriented microtubules represent the median bodies (arrows) and flat rows of microtubules adjacent to central pair of axenomes are portions of the axostyle. Fragments of the striated disk are also present.*

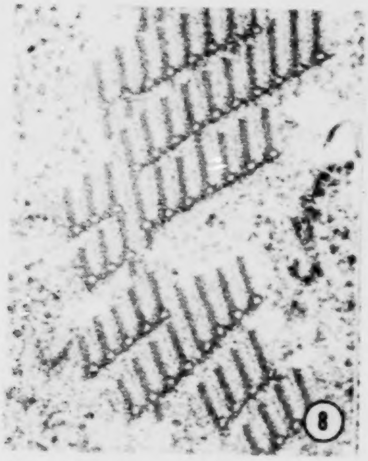

**FIG. 8. Giardia lamblia** ***cyst. 52,800X. Cross-sectioned fragments of the striated disk. Note cross bridges connecting the microtubules and the fine filaments between the ribbons. (Sheffield and Bjorvatn, 1977. Courtesy of American Journal of Tropical Medicine and Hygiene).***

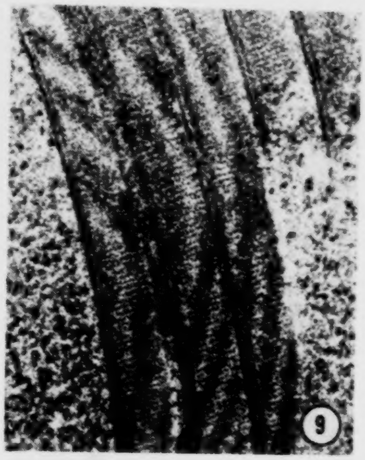

**FIG. 9. Giardia lamblia** ***cyst. 49,300X. Longitudinally sectioned striated disk fragments. Note periodicity in ribbon structure. (Sheffield and Bjorvatn, 1977. Courtesy of American Journal of Tropical Medicine and Hygiene).***

Although the median body has been considered a structure of the trophozoite by earlier workers, Sheffield and Bjorvatn(15) found a group of randomly arranged microtubules in the vicinity of the flagellar axonemes of *G. lamblia* and related it to the median body. The compactness of the microtubules was less than that seen by Friend(5) in *G. muris*, so the apparent absence in stained light microscope preparations may be due to an altered configuration during reorganization in the cyst. Short rows of microtubules, reported by Friend(5) to be ventral extensions of the median body, were also seen in *G. lamblia* cysts by Sheffield and Bjorvatn(15). However, the rows were not closely associated with the median body microtubules but commonly were seen coursing parallel and adjacent to two flagellar axonemes and were described as components of the axostyle. Comparable rows of microtubules were found in cysts of *G. muris* (Sheffield, unpublished observations) (Fig. 10). Occasionally, double rows of microtubules occurred on each side of the axonemes suggesting a possible duplication of the structure prior to division of the parasite.

Two previously undescribed structures were observed in *G. muris* cysts. The first consisted of a series of dense lines that were either straight or slightly curved and grouped together in a disorderly array of angles (Fig. 11). It is possible that they represent developing elements of the striated disk. The other structure appeared as a somewhat rounded mass occuring often at several places in the cytoplasm (Figs. 10, 12). Structurally, the mass had a

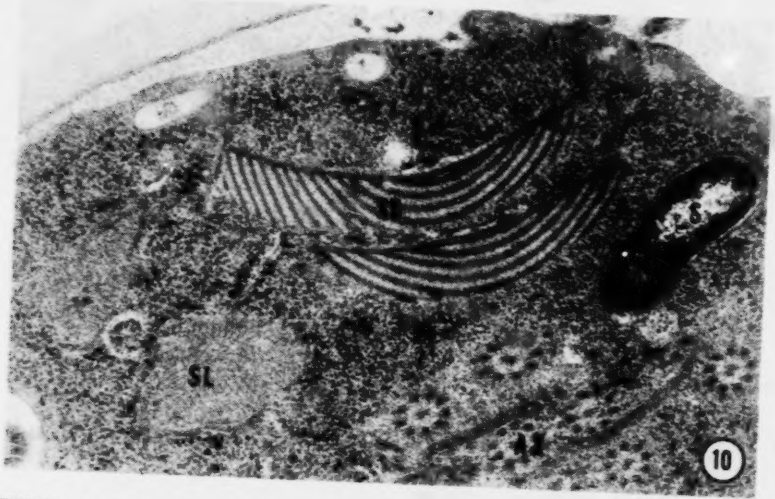

**FIG. 10.** **Giardia muris** *cyst. 35,600X. Striated layer of ventrolateral flange is present as are portions of striated disk and axonemes of flagella. Note bacteria-like endosymbiont in cystoplasm.*

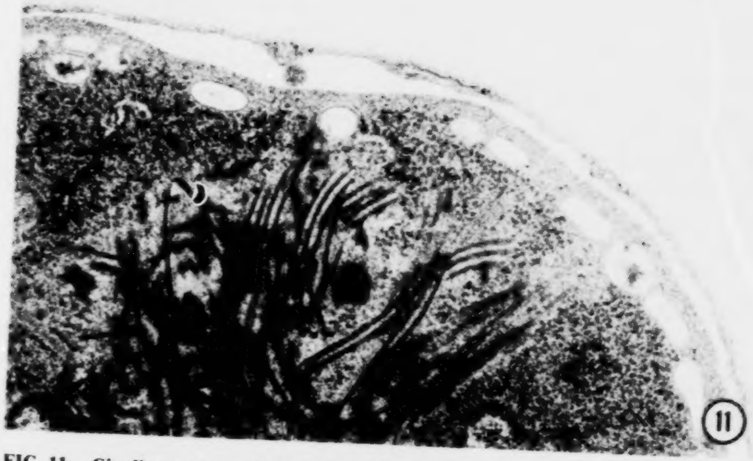

**FIG. 11.** **Giardia muris** *cyst. 34,700X. Randomly arranged dark lines (arrow) may represent forming elements of the striated disk. Dense amorphous structures are often seen in association with the dark lines.*

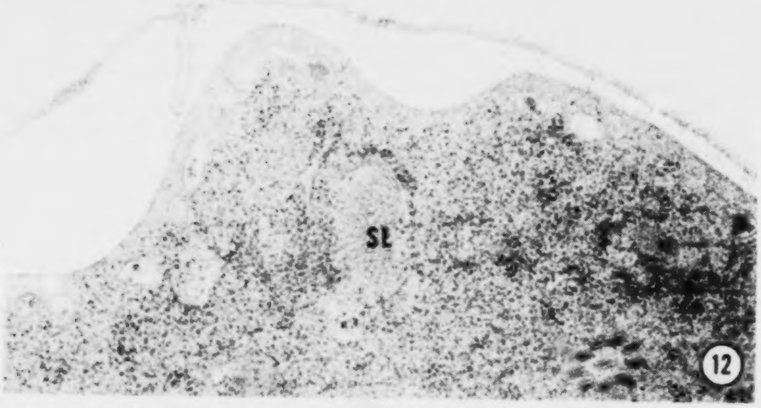

FIG. 12. **Giardia muris** *39,350X. Areas of light and dark lines with definite periodicity resemble the striated layer of the ventrolateral flange and may indicate assembly of this material in the cyst.*

definite periodicity produced by evenly spaced light and dark elements. When cut across these elements, a crystalline appearance was seen. The structure corresponds closely to that of the striated area of the ventrolateral flange demonstrated by Friend(5) in *G. muris:* Whether the structure is a remnant from the trophozoite or a newly formed component is not known.

Undoubtedly, the electron microscope has provided us with much information on the substructure of *Giardia*. At the same time, new questions have arisen. While the cytological aspects of the parasite have been well described, the functional ones need further study. It is hoped that the recent increased interest in giardiasis, as evidenced by this symposium, coupled with new techniques to provide experimental infections and large numbers of parasites will facilitate elucidation of the functional role of the various organelles presently known.

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## Discussion

F. SCHAEFER: With regard to cyst vacuolar areas, were those perhaps artifacts around the shell of the cyst wall due to flotation techniques that might have been used in preparing the cysts for electron microscopy?

H. SHEFFIELD: That is a very appropriate question in view of our earlier discussion of sucrose and flotation problems. I do not believe that these spaces are really caused by different isotonic pressures of flotation solutions. We see them in different cysts within the same preparation. In the same EM section you may see cysts in which the cytoplasm is very closely applied to the cyst wall, and in other cases where there are large open areas in between.

All of these open spaces, or lacunae, are all lined with a membrane, and we think if there had been a shrinking or a pulling of cytoplasm apart in some way we would not find any membrane around it.

D. JURANEK: Are there any ultrastructural differences between *Giardia lamblia* and *G. muris*?

H. SHEFFIELD: I really cannot report any differences at this point. We have not looked at a large number of *G. muris* cysts, and generally speaking, they look quite similar, but I would hate to say that they are different or similar.

S. ERLANDSEN: One comment about the activity of the flagella that I think needs to be kept in mind. In Dr. Holberton's work he was using quartz halogen lamps for illumination, and I think most of the trophozoites that he was observing were rather sick and that is why only the ventral flagella were actually beating. All of the flagella beat with a high frequency, with the exception of the caudal pair which appear to be inactive. This flagella activity may be very important in terms of attachment to the surface.

Have you seen any structure close to the nuclei that might be associated with the production of material within the lumen of the vacuoles or vesicles located near the surface?

H. SHEFFIELD: We have not observed vesicles which you might relate to Golgi vesicles in the ventral area of the parasite. The vesicles seen in my experience are always around the periphery, and I have never seen similar structures internally. Around the naked area on the ventral surface they look very similar.

# Surface Morphology of *Giardia* Cysts Recovered from a Variety of Hosts

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## ABSTRACT

The surfaces of *Giardia* cysts, collected from feces of laboratory mice, guinea pigs, dogs, mule deer, and humans, have been studied with scanning electron and phase microscopy. The cysts appear to be elliptical and slightly asymmetric, measuring approximately 10 by 6μm. The surface was relatively smooth without any characteristic features and cysts from all hosts appeared to be indistinguishable.

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*Giardia lamblia* has been known by several names for more than 100 years. Organisms possessing the exceptional morphological features of this species have been identified from the following hosts: human (1), cat (1,2,12), dog (3,4), horse (5), cattle (1,6), non-human primates (1,7), sheep and goat (1), wild and laboratory rats and mice (1,8,9), rabbits (1,10,12,13), guinea pig (1,11), chinchilla (1,12,13), and hamster (1). *Giardia* from these hosts have been variously considered to represent the same or a different species and have been named accordingly (1,14).

Based on clinical impressions, a potential for pathogenicity in human hosts had been long adduced for this organism. However, until this decade *Giardia* did not appear to represent a significant pathogen in the United States although the medical practice of eastern European countries had reported individual infections and recognized epidemics in institutionalized populations for a much longer period.

The reported outbreaks of *G. lamblia* infections in the United States, with evidence for importation and transmission of the organism by municipal water supplies, have provoked questions about the source of these infections, the presence of a reservoir in nature (15), and the methods of detecting small numbers of *Giardia* cysts in large quantities of water (14).

In recognizing the source of *Giardia* infections, considerable assistance would be provided if one or more of the distinguishing characteristics of *Giardia* cysts from a given host could be identified, especially if those hosts reasonably could be inferred to contaminate water supplies. The present work has examined the surface morphology of *Giardia* cysts, obtained from several hosts, with the prospect of identifying any distinguishing surface features.

## MATERIALS AND METHODS

Fecal samples from humans were received from laboratories in Virginia and South Carolina. All samples from animals were obtained through the courtesy of Dr. Charles Hibler, Colorado State University, Fort Collins, with the exception of the laboratory mice sample which was obtained through the courtesy of Dr. Robert Russell, Naval Research Laboratories, Bethesda, Md. Some samples were mailed after being suspended in 10% formalin solution. Alternately, specimens were obtained as fresh material and then fixed (in formalin or glutaraldehyde) or mixed to form a slurry in tap water when processed soon after collection. An aliquot of 0.5 to 1.0ml of these supensions was initially passed through three Kimwipes to remove large particulate matter. Then the solution was filtered through a 20$\mu$m pore, 47mm diameter polyethylene membrane (Tetko, Inc.) in a standard steel parabola filtration funnel. One drop of this filtered solution was usually removed and examined by phase contrast microscopy. Finally, a syringe was used to pass this filtered solution through 13mm diameter, polycarbonate membranes (Bio-Rad Laboratories) in two Swinney filters mounted in series, an 8$\mu$m pore membrane preceding a 5$\mu$m pore membrane. The filtrate was discarded. Additional fixation and dehydration solutions were passed through the membranes as needed using a syringe.

If the cysts and fecal matter were not already fixed, 5ml of 2.5% glutaraldehyde were slowly passed through the membranes. The material collected on the membranes from all specimens was then osmicated by adding 2ml of 1% osmium tetroxide with the syringe and slowly filtering the solution through the membrane over a period of 20 minutes. The membranes were then rinsed with 5ml cacodylate buffer. For dehydration 5ml of 50% ethanol were added, 2ml being filtered through the membranes to insure contact with the sample. After a ten minute contact period, the remaining ethanol was filtered through the membranes. The dehydration was continued similarly with increasing concentrations of 75, 95, and 100% ethanol. After the last ethanol solution had passed through the membranes, the Swinney holders were disassembled and the membranes carefully removed and air dried for two minutes. The dried membranes were attached to 13mm scanning electron microscope (SEM) stubs with double-stick tape, then coated with gold in preparation for viewing in the SEM.

Some specimens were also critical-point dried rather than air dried. After the 100% ethanol had filtered through the membranes, the Swinney holders were opened quickly, the membranes immediately placed on 13mm steel membrane holders in 100% ethanol, and a 3$\mu$m pore polycarbonate membrane placed directly on top of each 5$\mu$m or 8$\mu$m pore membrane. The membrane holders were secured, quickly placed in the critical-point drier, and dried using carbon dioxide as the transition fluid.

Examination of the gold-coated membrane was made in the ETEC scanning electron microscope located at the Bureau of Biologics, Food and Drug Administration, Washington, D.C.

## RESULTS

### Laboratory Mouse

Fecal pellets from a laboratory strain of mice with chronic giardiasis provided large concentrations of cysts. Fig. 4 and 5 show typical examples of cysts in phase microscopy. No other cyst-like forms were seen showing the size and well-known internal features of *Giardia.* Figs. 1, 2, and 3 show the corresponding structures as observed on a 5μm pore membrane in the SEM. A relatively clean and high density collection of these forms was obtained from the fecal specimens. Structure dimensions were 9.0 by 6.0μm giving a length/width ratio (L/W) of 1.50. All considerations of shape and size, including recovery from the membrane and re-identification of the internal morphology by phase microscopy, indicated that the structures seen in SEM were, in fact, those of *Giardia* cysts.

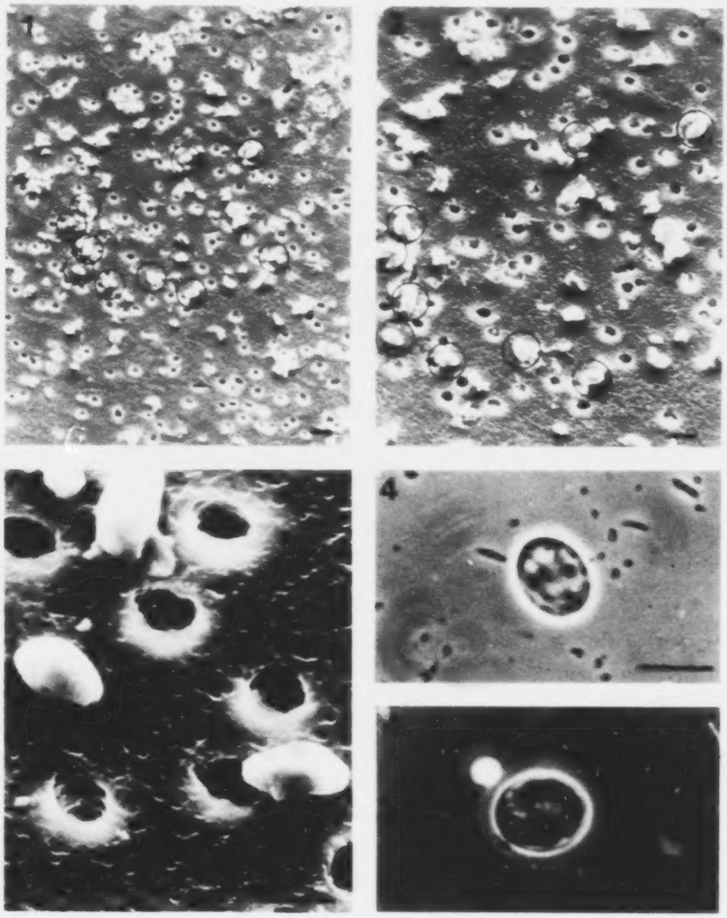

FIG. 1-5. **Giardia** *cysts from the mouse. Fig. 1 shows ten* **Giardia** *cysts prepared from feces of the laboratory mouse and photographed on a 5 μm pore membrane at 450x, and in Fig. 2 at 750x. The three cysts at the lower left are photographed again in Fig. 3 at 3000x. Two phase micrographs of similar cysts from the same source are shown in Fig. 4 and 5 at 2000x. Scale markers are 10 μm in all photographs unless noted to the contrary.*

## Guinea Pig

After processing, the guinea pig feces yielded a noticeably lower concentration of cysts than those from the laboratory mouse; however, the cyst could be located and identified by phase microscopy (Fig. 9) and the corresponding structure was seen in SEM (Fig. 6,7, and 8). The cysts were the expected elliptical shape and approximately 10 x 6μm (L/W = 1.66). The cysts appeared slightly asymmetric. No consistently occurring pores or projections were present, but the cyst walls showed signs of cracking, the possible consequence of chemical fixation and/or age of the cysts.

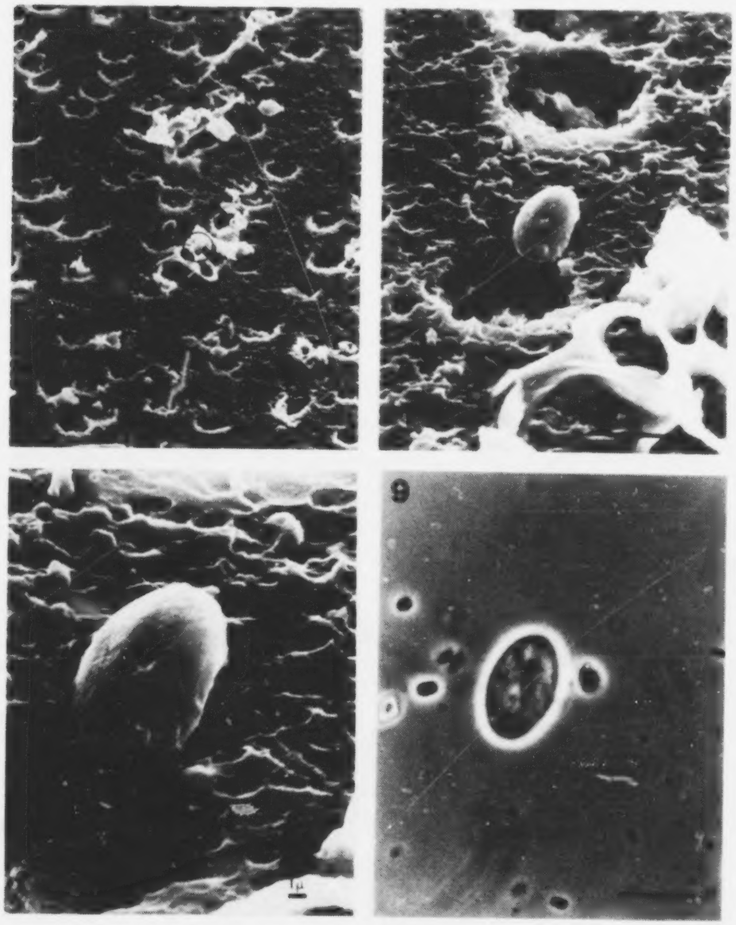

FIG. 6-9. **Giardia** *cysts from the guinea pig. Fig. 6 shows three cysts on an 8 μm pore membrane photographed in the SEM at 750x, in Fig. 7 at 3000x and in Fig. 8 at 7500x. A similar cyst from the same source is photographed at 2000x in phase microscopy in Fig. 9.*

### Dog

Cysts found in dog feces were noticeably variable in dimensions. Fig. 13 and 14 show phase micrographs of cysts with the dimension 13 by 11μm (L/W = 1.18) and 12 by 10μm (L/W = 1.20). These two micrographs show that a larger, less elliptical cyst is present together with a smaller, more elliptical cyst. Fig. 10 and 11 show the more elliptical structure as seen in SEM with a higher magnification given in Fig. 12. There are no distinguishing surface features although the more spherical cyst appears to have a smoother surface than the more elliptical type. The elliptical type is slightly rougher and also more like the cysts from the guinea pig and mouse.

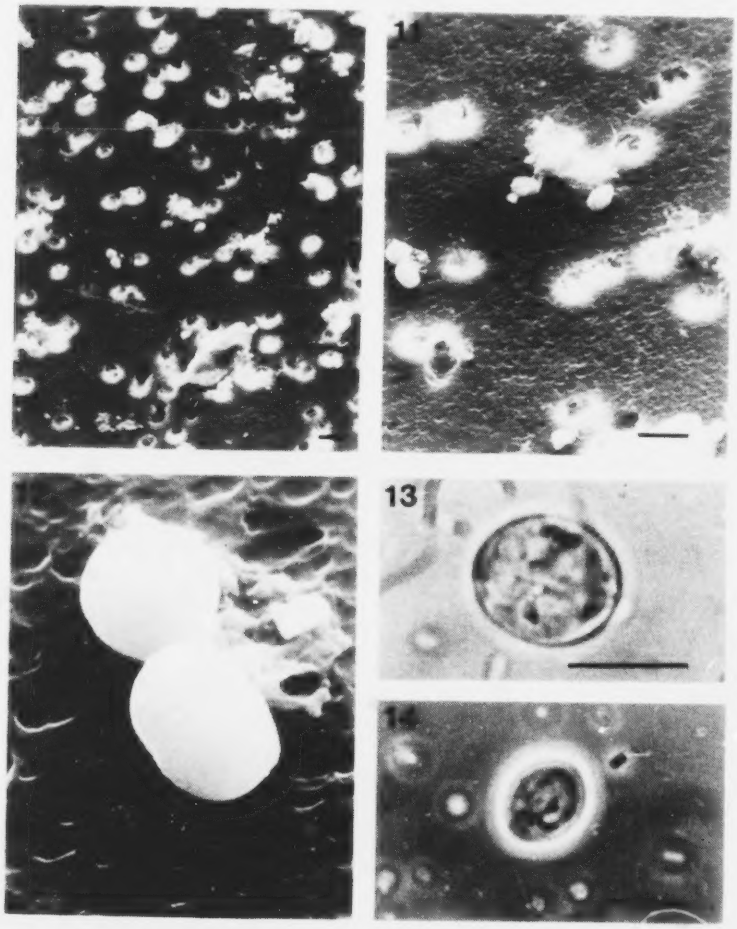

**FIG. 10-14.** **Giardia** *cysts from the dog. Fig. 10 shows three cysts on a 5 μm pore membrane at 750x, in Fig. 11 at 1500x, and Fig. 12 at 10,500x. Two similar cysts from the same source are shown in phase microscopy at magnifications of 4000x and 2500x in Fig. 13 and 14, respectively.*

## Mule Deer

*Giardia* were extracted from feces of this host in relatively high concentration and were predominately found on the 5$\mu$m pore membrane (Fig. 15,16,17). The cysts were identified in phase microscopy, although no photographs are available. The 9 by 6$\mu$m size and elliptical appearance are similar to those from the mouse, guinea pig, and dog. The walls of the cysts show some of the rough and flaking surface observed on previous cysts, and again no unique morphological features were noted.

On the membrane at the base of approximately a fifth of the cysts, material was noted which appeared to have melted, flowed evenly from the

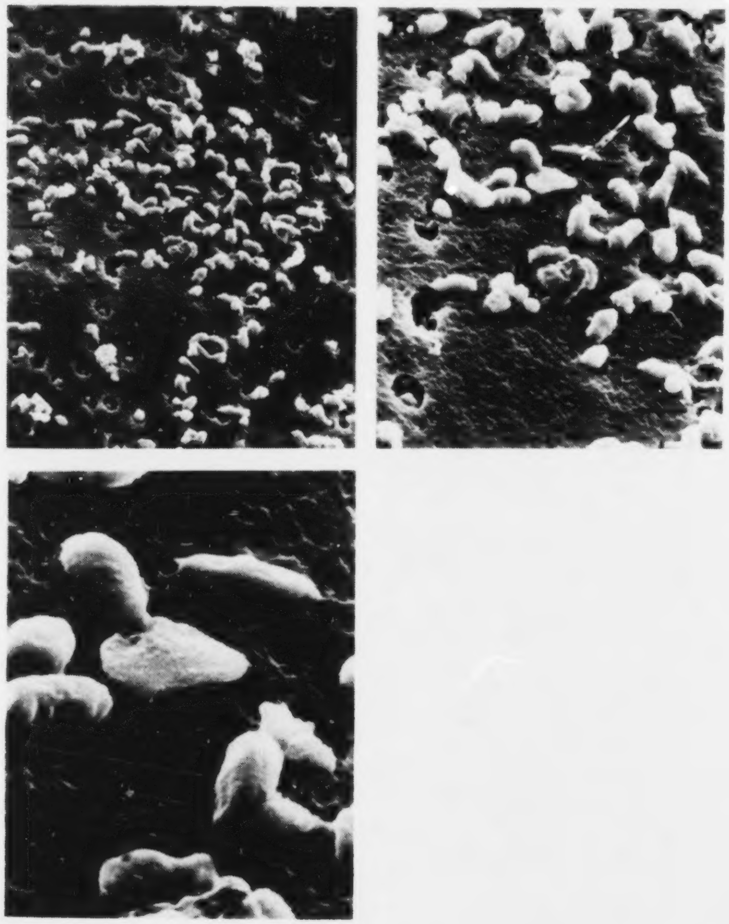

FIG. 15-17. **Giardia** *cysts from the mule deer. Fig. 15 shows over fifty cysts prepared from feces of the mule deer and photographed on a 5 $\mu$m pore membrane in the SEM at 750x in Fig. 16 at 1500x, and in Fig. 17 at 4500x.*

cysts, and solidified forming a disk around the point of attachment between the organisms and the support membrane. Such rings are noticeable on several of the cysts in Fig. 17. Even after this unexplained denuding no new features are evident on the surface of those cysts. No attempt was made to repeat this observation; thus its significance is unknown.

On Fig. 15 and 16 several spherical structures are apparent. These are possibly cysts of other protozoa, which possess smooth surfaces and are between 5 and 6$\mu$m in diameter. If a slightly different procedure is followed in collecting the filtrate prior to passage through the Swinney filters, a higher

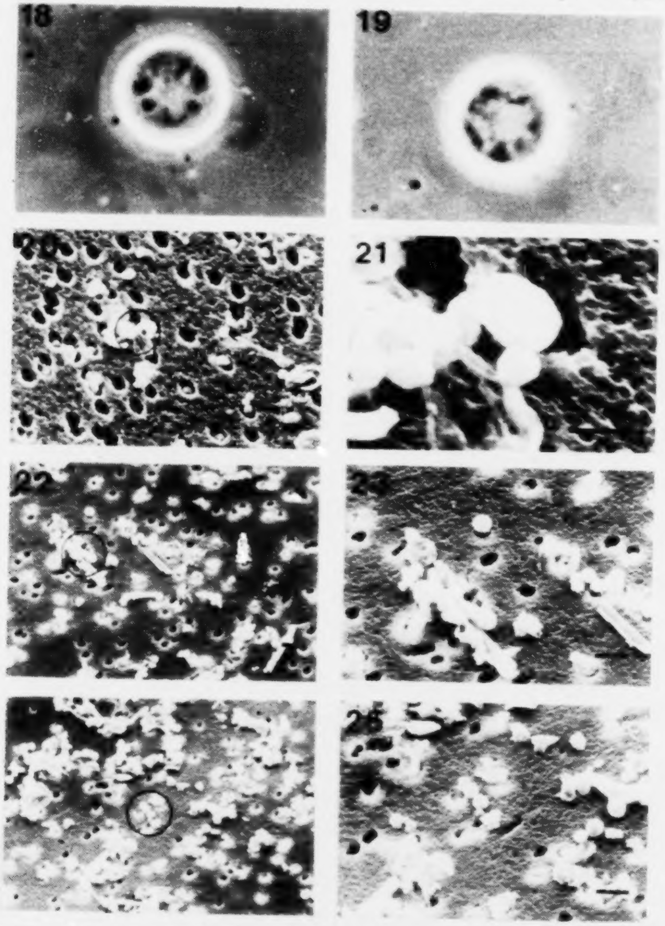

FIG. 18-25. *Unknown cysts from the mule deer. Figs. 18 and 19 show phase micrographs of two unknown cysts from the feces of the mule deer which have some morphological similarities to* Giardia. *They are also shown in Figs. 20 and 21 on an 8 $\mu$m pore membrane and are photographed at 750x and at 1500x respectively. In the last 4 micrographs they are on a 5 $\mu$m pore membrane and photographed at 750x in Figs. 22 and 24, and at 1500x in Figs. 23 and 25.*

concentration of these structures is noticed with fewer *Giardia*. Two of these cysts are shown on Fig., 18 and 19 which clearly demonstrate that they are not *Giardia*. Fig. 20 illustrates the collection of cysts and debris on the 8μm pore membrane, a portion of which is enlarged in Fig. 21 to show four of the unknown cysts and a single *Giardia* cyst. The accumulation of cysts and solid fragments on the 5μm pore membrane are shown on Fig. 22 and 24 with areas magnified in Fig. 23 and 25.

### Human

Four phase micrographs (Fig. 28,29,30, and 31) of *Giardia lamblia* cysts are shown which illustrate a morphology similar to those already noted from previous hosts. Fig. 26 and 27 are scanning electron micrographs of cysts which are typical of those collected on both the 8 and 5μm pore membranes, but the anticipated elliptical shape is not found. Rather, the cysts appear to have become distorted, creating a shape which is more cuboidal than elliptical. This is the only example where the results obtained by scanning and phase microscopy are not complementary.

### Non-Giardian Cysts

Three test protozoans were examined as internal controls to provide confidence that the SEM techniques being employed would indeed demonstrate pores, ridges, and surface indentations if such were present on the cysts of *Giardia*. The three organisms chosen were *Naegleria*, *Acanthamoeba*, and *Sarcocystis* which have particular relevance to this study because their cysts may be isolated from water.

*Naegleria fowleri* is shown in Fig. 32,33 and 34 with micropores, 0.1μm in diameter, and clearly evident surface indentations. *Naegleria gruberi* is photographed in Fig. 35,36, and 37 with a large depression on a mature cyst and micropores clearly evident on the walls of the empty cysts.

*Acanthamoeba culbertsoni* is photographed in Fig. 38 to 42 and shows quite clearly the ratchet-like internal structure which is conspicuous following the collapse of the cyst wall. When the cysts are critical-point-dried and the walls do not collapse such internal structures are not evident.

Finally, *Sarcocystis sp.* is shown in an air dried preparation in Fig. 43 and 44, in a critical-point-dried preparation in Fig. 45, and in phase microscopy in Fig. 46 and 47. The slightly raised ribbing along the surface of the cyst wall, forming an oval hexahedron, is easily and consistently observed with the SEM.

## DISCUSSION

The examination of *Giardia* cysts from five hosts, (mouse, guinea pig, dog, mule deer, and humans), demonstrated a degree of similarity in reference to size, and appearance. The cysts appear as elliptical, slightly asymmetric structures measuring approximately 10μm by 6μm. The one exception, from humans, deviated from the elliptical shape of cysts when examined in the SEM but not when examined in phase microscopy. This size alteration was very probably a consequence of some procedural error which was

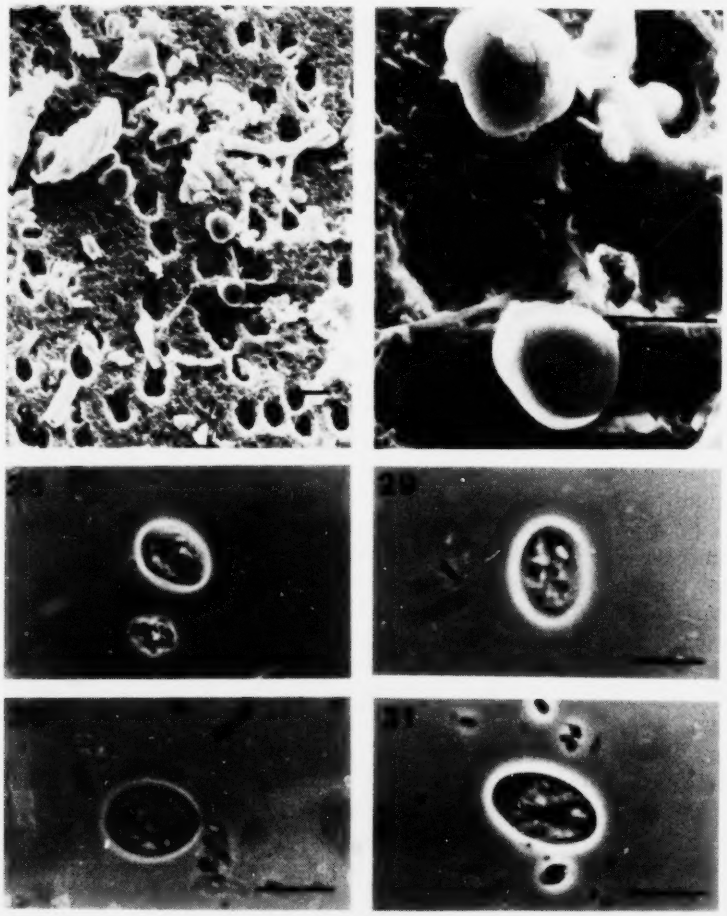

FIG. 26-31. Giardia *cysts from humans. Fig. 26 is an SEM micrograph of several* Giardia *cysts from human material on an 8 μm pore membrane at 750x. Fig. 27 is the same material magnified to 3000x. Figs. 28, 29, 30, and 31 are phase micrographs of cysts of the same sources at 2000x.*

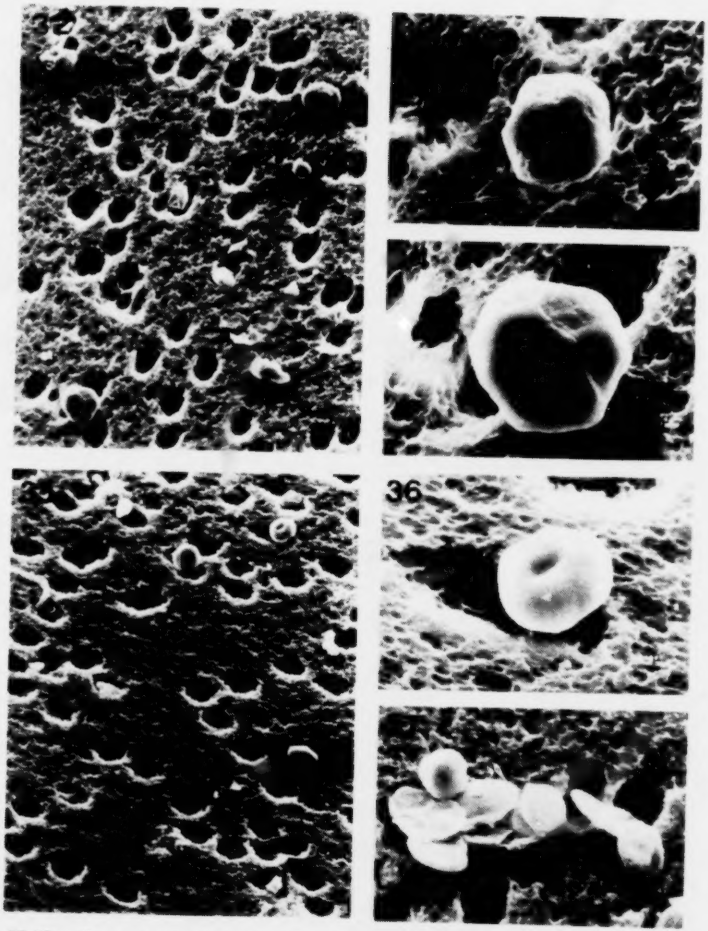

FIG. 32-37. *Cysts of two* Naegleria *species. Cysts of* N. fowleri *(ATCC 30463) are shown in Fig. 32 on an 8 μm pore membrane at 750x and in Figs. 33 and 34 at 3000x. The initial photograph shows a typical field with two relatively intact and numerous collapsed cysts. The large cyst in the lower left corner is shown enlarged in Fig. 34 which along with Fig. 33 show micropores and surface irregularities. Cysts of* N. gruberi *(ATCC 30133) are shown in Fig. 35 on an 8 μm pore membrane at 750x, in Fig. 36 at 3000x, and in Fig. 37 at 1500x. The cyst in the upper right corner of Fig. 35 is shown in Fig. 36, and a group of collapsed cysts are shown in Fig. 37.*

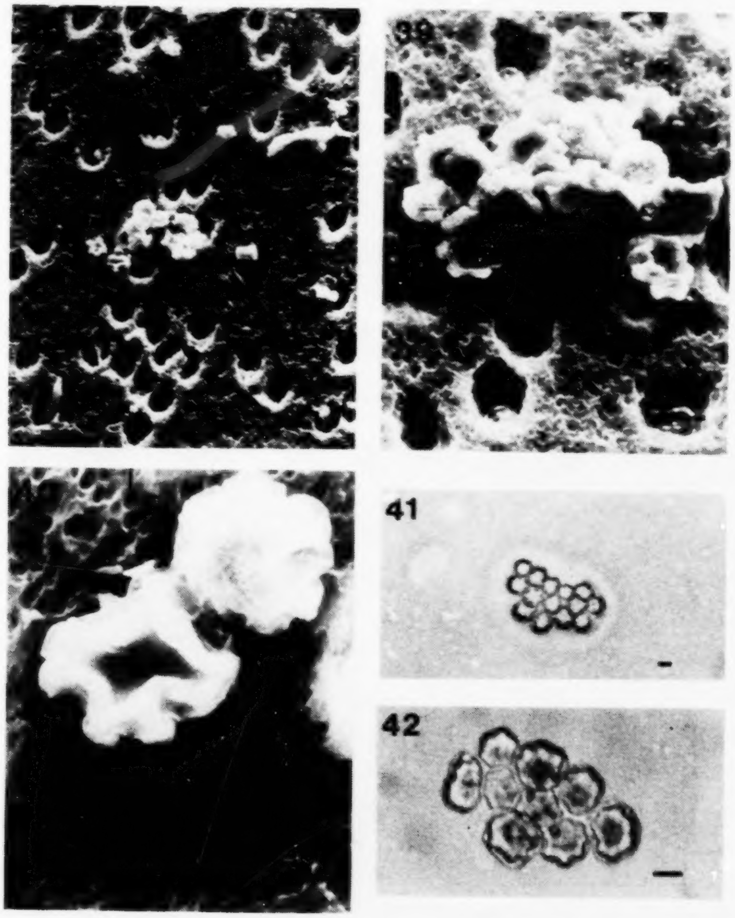

FIG. 38-42. *Cysts of* Acanthamoeba culbertsoni *(ATCC 30172). Numerous cysts are seen in SEM on an 8 μm pore membrane at 750x in Fig. 38. A similar group of cysts at 1500x is shown in Fig. 39, and a mature cyst (left) together with a rounded, less mature cyst is shown at 8900x in Fig. 40. Figs. 41 and 42 show the appearance of the two groups of cysts* in situ *on the surface of an agar plate as they appear in phase microscopy at 300x and 600x, respectively.*

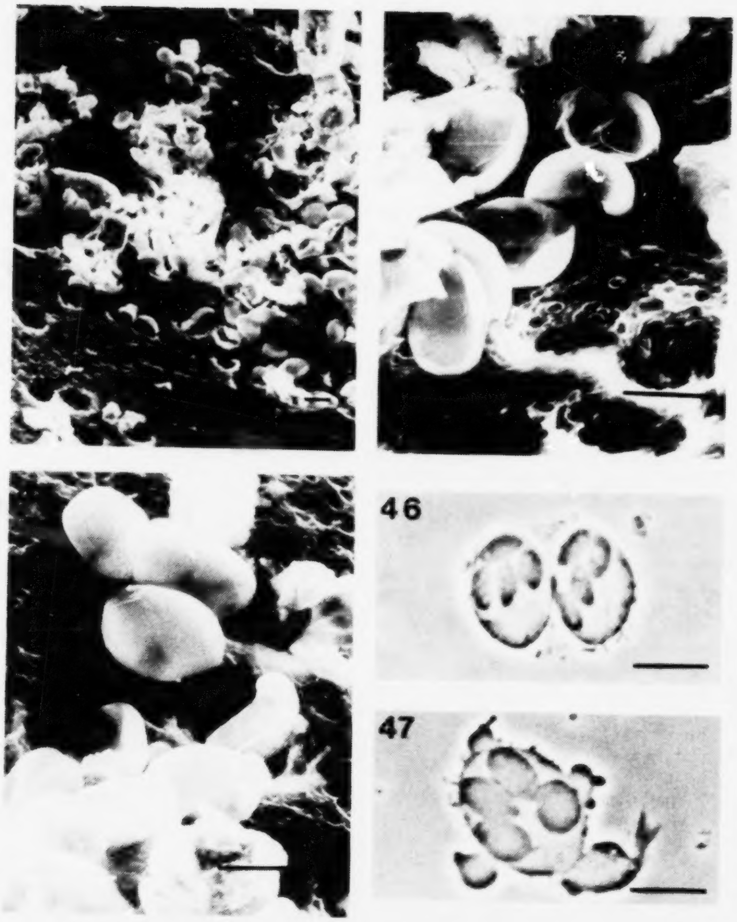

FIG. 43-47. *Cysts of* Sarcocystis sp. *Fig. 43 illustrates a large number of cysts at 750x. In Fig. 44 most of the cysts have collapsed during air drying in the preparation procedure. Figs. 44 and 45 are of the same magnification (3000x) but the cysts in Fig. 45 were prepared by critical-point-drying which demonstrates improved retention of the cyst shape. Figs. 46 and 47 are phase micrographs at 2000x. The pattern of surface ribbing which stands out sharply in the scanning electron micrographs is faintly delineated in the phase micrographs, especially in Fig. 46.*

unknowingly repeated in each human sample and is not felt to represent a biologically significant morphological characteristic. Research is underway to clarify this matter.

The surface of all *Giardia* cysts is relatively smooth and even, without a consistent pattern of pits, pores, or major depressions and without ridges, projections, or a uniform pattern of granularity or roughness. This surface smoothness must then be the major surface characteristic of the organisms. Therefore, based on our observations of the surface of cysts, we cannot support the hypothesis that *Giardia* from each host represents a separate species. Consequently, it appears impossible at this time to determine the source of a sample of cysts through an examination of the cysts with the SEM. The possibility still exists, however, that with some modification of the procedure for the detection of nematodes in drinking water (16) it will be possible to detect *Giardia* cysts with the SEM in samples of water.

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## Discussion

R. OWEN: Did you do transmission electron microscopic correlation on any of your filter material?

A. TOMBES: No, we have not.

R. OWEN: Dr. Erlandsen has described various ways of doing this. It really is critical because unfortunately with the scanning microscope, when you are looking at the surface, little round things look like little round things. The stool has a lot of little round things in it, and its really hard to tell one from another. It is essential before making any judgment about seeing anything in scanning microscopy that you take the specimen and look inside. You can take it off the stub even with gold on it, embed it in resin, and section it as though it were cultured material. It is just like not telling books by their covers.

The other problem that we have had is looking at material that has been dehydrated, particularly on a filter. The evaporation rate of alcohol is so fast that when you take your filter material and try to get it in your critical-point dryer it tends to air dry before it gets there. Even though you go through the critical-point drying procedure, essentially you have an air dried specimen.

Did you ever take the material out of the filter holder under alcohol and keep it under alcohol continuously before putting it into the critical-point dryer?

A. TOMBES: No, we did not.

R. OWEN: It is very hard to avoid air drying your material, and there may be some surface characteristics in *Giardia* that may be lost. This is unlike nematodes where you have enough size that they retain enough moisture to keep from drying the wrong way.

A. TOMBES: I realize that situation. Commenting on the second question first, if you are quick you can remove the membrane, keeping it under alcohol while you move it to the dryer. With the Bomar critical-point dryer you can move the membrane within 3 or 4 seconds so that it does not air dry. Holding the membrane properly, you slip it onto a steel support that will hold it in the alcohol while you secure the retainer during the drying process. Consequently, I believe this eliminates the problem of unintentionally air drying the cysts.

We saw with *Sarcocystis* that different cyst morphologies are observed following air or critical-point drying. This is not the case with *Giardia* where no consistent differences were observed between the 2 drying procedures.

R. OWEN: From your continuous correlation with phase microscopy, I presume that what you are looking at are *Giardia* cysts, but I would feel much better if at least once you had a transmission correlation so you could be sure.

A. TOMBES: We have been devoting most of our time to scanning and phase microscopy. If we go to TEM and section some of the material, it may strengthen our belief that we have *Giardia*, but it would not help us in deciding whether we have *muris* or another species and that was the objective of our study.

S. ERLANDSEN: In most of the pictures there seemed to be a tremendous amount of secondary emission coming off the cyst, and essentially it was whited out on the screen. Have you used lower voltage scanning microscopy to see surface detail?

A. TOMBES: Yes. In the dog cyst for example, the smooth one, you would suspect that any morphology there would be lost because of the secondary emission. We lowered the voltage on a number of occasions, but then you lose detail. We have also altered the photographic settings on the microscope to try to improve the detail.

J. HOFF: Many of us seem to have the need for obtaining good clean cyst preparations. My question is one of general inquiry as to a possible way of doing this for our own work in disinfection. We need highly purified suspensions of cysts that retain viability and are free of both soluble and particulate material that may exert a chlorine demand.

There is a recently developed centrifugal procedure called elutriation which involves low speed centrifugation. The fluid flows in a direction counter-current to the centrifugal force, and some quite remarkable separations of cell populations with different surface characteristics have been achieved. I wonder if anyone has used this procedure?

A. TOMBES: I have not used the procedure. Has anyone here used the procedure?

(No audible response.)

T. ERICKSEN: Do you think that the presence of the 10 per cent formalin in your fecal specimens might have had an effect on your cysts? There has been experience with these under storage, particularly at room temperature, where the cysts will disappear. Secondly, it is my understanding that you said the cysts did not have pores. Is that correct?

A. TOMBES: That is correct, and is contrary to what we had talked about earlier. The explanation is that we must have had an artifact in the preparation of an earlier human specimen to give us the morphology that we were seeing at the time.

I am fairly certain that some of the fine cracking or flaking that is evident on the *Giardia* cyst in the SEM is a consequence of the fixation procedure. We are in the midst now of a study to determine the effects of shelf life on the cysts in a variety of conditions to see if the 10% formalin does create distortion with time, and what effects 2% glutaraldehyde might produce.

V. OLIVIERI: Do you have any information on the per cent recovery for the various steps in your procedure? Also, how many samples of human and animal fecal material have you examined?

A. TOMBES: We have collected fecal material from eight mice, one guinea pig, one dog, one mule deer, and three humans. When working with sucrose gradients, we found a 15 or 20% recovery of cysts on the membrane. We have not conducted a similar study using the procedure presented today. I am sure we are losing many cysts in the Kimwipes and on the 20 $\mu$m pore screens; however, the objective of the study was to look at the cyst surface and not try to determine the per cent recovery.

R. RENDTORFF: The micromanipulator made by Dr. Charles Reese of NIH is a very simple technique to get cysts in very minute quantities of water. In terms of number, in our studies, we went up to concentrations of over a million. Those were not free of particulate matter because we did not use the micromanipulator. We did, however, obtain accurate numbers of cysts up to 100 in small amounts of water very easily. I think sometimes modern techniques are perhaps too refined.

A. TOMBES: There is no problem in getting a hundred cysts under a phase microscope, and there is no problem in getting similar cysts at about the same density on the membranes in the SEM. The problem concerns picking up one cyst which is unquestionably *Giardia lamblia* or *muris*, and then looking at that same cyst in the SEM.

R. RENDTORFF: I really think that could be done.

A. TOMBES: I do not doubt that it can be done, and if I could get a micromanipulator to use, I would do it immediately.

R. WIDDUS: I can put you in touch with people that can actually do the micromanipulation and have been working on it.

A. TOMBES: This is a very important point and goes back to what Dr. Owen was saying at the beginning. That is the test, to know what we are looking at in the SEM. I am the first to admit that the data are circumstantial for we have been correlating phase microscopy of the cysts with what is seen in the SEM.

# SESSION II - THE DISEASE

*Chairman - Walter Jakubowski,*
*U.S. EPA, HERL, Cincinnati, Ohio*

## Managing the Patient with Giardiasis: Clinical, Diagnostic and Therapeutic Aspects

**M. S. Wolfe**

## The Possible Use of an Indirect Immunofluorescent Test Using Axenically Grown *Giardia lamblia* Antigens in Diagnosing Giardiasis

**G. S. Visvesvara and G. R. Healy**

## The Experimental Transmission of *Giardia lamblia* Among Volunteer Subjects

**R. C. Rendtorff**

## Giardiasis in the Mouse: Clues to Host Immune Mechanisms

**D. P. Stevens**

# Managing the Patient with Giardiasis: Clinical, Diagnostic and Therapeutic Aspects

**Martin S. Wolfe**

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## ABSTRACT

*Giardia lamblia* is the most common pathogenic intestinal parasite in the United States and is a leading cause of morbidity in travelers returning from abroad. Clinical features may differ in the recently acquired acute stage and in the established subacute and chronic stages. Diagnosis can usually be made with three well performed stool examinations, properly collected on alternate days, utilizing both direct smear and formol-ether techniques. Examination of duodenal contents and intestinal biopsy only rarely confirm the diagnosis when these stool examinations are negative. Not infrequently, giardiasis is suggested by typical symptoms and exposure history, and parasitologic confirmation cannot be made after reasonable diagnostic effort. In this situation, an empiric trial of the drug quinacrine often leads to gratifying clinical improvement. Quinacrine is the treatment of choice, with metronidazole and furazolidone being alternative available drugs in the United States. All three drugs, however, have potential problems associated with their use.

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*Giardia lamblia* is the most common pathogenic intestinal parasite in the United States, according to the most recent Intestinal Parasite Surveillance Report of the Center for Disease Control(1), and is also the most frequently encountered intestinal pathogen in my practice in Washington, D.C. Over a five year period from late 1969 to late 1974, 670 cases of giardiasis were found at the Office of Medical Services of the Department of State, while during the same period 508 cases of amebiasis were diagnosed. These 670 giardiasis cases, occurring primarily in returnees from 97 different countries, and in a much smaller number of individuals who had not left the United States, have been reviewed and are the primary source of data presented here.

## CLINICAL FEATURES

In experimental infections, the prepatent period, based on the first detection of parasites in the stool, averaged 9.1 days(2). The most reliable data on time interval between infection and onset of acute symptoms comes from investigations of travelers returned from the Soviet Union. The mean time period until the onset of illness from entrance into the Soviet Union has been 12 to 15 days (ranging from 1 to 75 days)(3,4,5).

Symptomatology in giardiasis differs, depending on the duration of infection when diagnosis is first made.

### Acute Stage

Typically, there is the sudden onset of explosive, watery, foul diarrhea; marked abdominal distention, foul gas, and belching; and nausea, anorexia, vomiting, fatigue, and cramps which are usually upper or midepigastric.

Lowgrade fever and chills may precede or occur with the onset of diarrhea, as may headache. Mucus occurs only rarely in the stool and when blood is present in the stool it is invariably secondary to anal irritation from the diarrhea. Again, some of the best available data on the symptomatology of acute infections comes from investigations of individuals recently returned from the Soviet Union. Table I shows the frequency of symptoms in a series of 32 of these cases with *Giardia lamblia* found in the stool(3).

The acute stage usually lasts only three to four days and is often not recognized at the time as being due to giardiasis. In some cases, the acute stage may last for months, leading to malabsorption, debility, and significant weight loss. This latter situation appears to be more common in young children than adults and perhaps explains why giardiasis had formerly been considered to be primarily a disease of childhood. The acute symptoms of giardiasis may mimic those of acute amebic dysentery, bacillary dysentery, bacterial food poisoning, and "travelers' diarrhea" caused by enterotoxigenic *E. coli.* But the foul-smelling stool, flatus, and belching, the marked abdominal distention, and the rarity of mucus and blood in the stool, are more characteristic of giardiasis.

## Subacute And Chronic Stage

Acute infections can develop into long-standing subacute or chronic infections. In returnees from residence or lengthy travel abroad, the acute

*Table I. Clinical features of 32 patients recently returned from the Soviet Union (3) with proven giardiasis compared with 105 State Department cases with undetermined duration of proven infection*

| Symptom | State Dept. % (n=105) | USSR % (n=32) |
|---|---|---|
| Flatulence | 46.7 | 56.5 |
| Foul Stool | 44.8 | 52:2 |
| Cramps | 32.4 | 59.4 |
| Distention | 31.4 | ---- |
| Anorexia | 20.0 | 56.2 |
| Nausea | 20.0 | 59.4 |
| Weight Loss | 18.1 | ---- |
| Belching | 15.2 | 30.4 |
| Heartburn | 14.3 | ---- |
| Headache | 11.4 | ---- |
| Constipation | 11.4 | ---- |
| Vomiting | 4.8 | 34.4 |
| Fever | 3.8 | 17.4 |
| Chills | 2.9 | ---- |
| Diarrhea | 62.9* | 71.9 |
| Blood in Stool | 0 | 0 |
| Mucus in Stool | 3.8 | 4.4 |
| Fatigue | 28.6 | 87.5 |

* 52.4% had soft or mushy stools; 10.5% had watery stools.

stage is often not recognized or recalled, and they frequently present with persistent or recurrent mild to moderate symptoms. In a series of 105 cases seen in 1974 at the Department of State with uncertain duration of infection, almost one-third to one-half presented with flatulence, mushy foul stools, primarily upper intestinal cramps, and abdominal distention. Other symptoms included anorexia, nausea, weight loss, belching, heartburn, headache, and constipation. Vomiting, fever, and chills occurred rarely. These symptoms are shown in Table 1 and are compared to the symptoms of the 32 acute cases. Type of stool passed, frequency of bowel movements, and virtual absence of blood, pus, and mucus in the stool for the 105 State Department cases from 1974, is shown in Table 2. Of these 105 cases, 39% were judged by our criteria to have mild symptoms (cramps, constipation, flatulence, foul stool, mushy stool); 41% were judged to have moderate degree of symptoms (above mild symptoms and/or belching, nausea, anorexia, vomiting, distention, heartburn, weight loss, fatigue, chills, fever and headache); and 6.7% were considered to have incapacitating severe symptoms (above symptoms plus frequent watery diarrhea, marked weight loss, and marked fatigue). No symptoms were present in 13.3% of this group. Thus 80% of these cases were considered to have mild to moderate subacute or chronic symptoms.

*Table 2. Characteristics of the stools of 105 State Department cases with proven giardiasis*

| | No. | % |
|---|---|---|
| A. Type of Stool | | |
| Formed | 35 | 33.3 |
| Mushy | 55 | 52.4 |
| Watery | 11 | 10.5 |
| Unknown | 4 | 3.8 |
| B. Blood | 0 | 0 |
| Pus | 0 | 0 |
| Mucus | 4 | 3.8 |
| C. Frequency | | |
| Normal | 65 | 61.9 |
| Increased | 22 | 21.0 |
| Very Frequent | 13 | 12.4 |

The typical non-acute stage patient presents with recurrent or less commonly persistent brief episodes of loose foul stools which may be yellowish, frothy, and float in the toilet water, accompanied by increased distention and foul flatus. Between exacerbations, stools are usually mushy or constipation may occur. Abdominal discomfort is often caused by the marked distention and when cramps occur they are usually in the mid to upper epigastrium. Sulfuric belching, known by Peace Corps Volunteers as the "purple burps," and substernal burning are not uncommon. Anorexia, nausea, and a gurgling or uneasiness In the epigastrium are frequent complaints, but vomiting is unusual in this stage. There may be tenderness to palpation in the right, and to a lesser extent, in the mid and left upper quadrants. These symptoms often suggest ulcer, hiatal hernia, or gall bladder disease. We have seen a few cases of urticaria associated with giardiasis, and others have reported this sign(6), erythema multiforme(7), and arthritic symptoms(8). Weight loss, lassitude or fatigue, headaches, and myalgias may occur. In rare cases, these symptoms may persist for years, but in the great majority of cases spontaneous disappearance of parasites and symptoms occurs after variable periods of time. If eosinophilia occurs, it is usually related to another concomitant cause.

Rare reports of cholecystitis(7,9) and pancreatitis(10) associated with the presence of *G. lamblia* parasites have been reported. Various types of malabsorption have been confirmed (11,12), including steattorhea, disaccharidase (particularly lactase and xylase) deficiency, Vitamin $B_{12}$ malabsorption, and protein-losing enteropathy.

A post-giardial lactose intolerance may develop, particularly in patients from ethnic groups with a predisposition to lactase deficiency, following apparent eradication of parasites with specific treatment. This must be considered in individuals with negative post-treatment specimens who have persistent mushy stools and excessive gas and distention, before administering further anti-*Giardia* treatment.

The duration of the asymptomatic cyst-passing state has not been determined.

## DIAGNOSIS

### Stool Examinations

In the majority of *Giardia* infections, a well-trained parasitology technician should usually make a diagnosis by stool examination. In our series of 670 parasitologically confirmed cases, utilizing direct smear and formol-ether concentration tests, 76% were positive on the first specimen, 90% were found with two specimens, and 97% were found with three specimens. An additional 1.7% were found on the fourth or subsequent specimen. The zinc sulfate flotation method in our experience has not significantly increased the positive yield over direct smear and formol-ether concentration methods. Examination of stools on alternate days has given us an increased positive yield over specimens examined on three consecutive days and this is probably related to the intermittent passage of parasites due to periods of their active multiplication. Some investigators claim that only 50% of proven cases can be confirmed by stool examination, but they usually perform either a single stool examination by formol-ether technique or they perform only direct saline smears without concentration(13).

Diagnosis appears to be easier in early acute than in established infections. In the acute stage, stools are frequently watery or loose and may contain only the more labile trophozoites, due to rapid bowel transit. It is thus important to either immediately examine a wet smear or to preserve stool in formalin, merthiolate-formalin, or polyvinyl alcohol (PVA) for later examination.

After the acute stage has passed stools are more often semi-formed or formed and contain the more hardy cyst form of the parasite. There is then no urgency to immediately examine "warm stools" and direct smear and formol-ether concentration examinations of unpreserved stools are often adequate to make the diagnosis. Purging does not appear to aid the diagnosis and routine culture methods are not presently available.

Not infrequently, we encounter returnees from abroad with a clinical picture highly compatible with giardiasis in whom we are unable to prove the presence of parasites by repeated stool examinations. It has been shown in some returnees to Finland following visits to the Soviet Union that in the first three weeks after infection, symptoms may be present before parasites become detectable in the stool and repeated examinations may be necessary to confirm the diagnosis(14). In given populations of *Giardia*-infected individuals in an endemic area with established infections, high, low, and mixed patterns of cyst excretion have been observed(15), and this situation may well occur in returnees from endemic areas. It has been suggested that positive confirmation of infection in low and mixed excretors might require examinations of two or three stools a week for four or five weeks, but this is tedious, expensive, and often impractical.

## Examination of Duodenal Contents and Biopsies

Where parasites cannot be found by stool examination in suspected cases, duodenal fluid examination may allow for their recovery. Our experience with duodenal tubal aspiration is limited, but this method has been reported to be more reliable than stool examinations in making the diagnosis(16). The "Enterotest", a gelatin capsule containing a string, has been shown by some workers to be a suitable substitute for duodenal intubation in obtaining *Giardia* trophozoites(17). Other workers suggest that examination of biopsies with Giemsa-stained sections and touch preparations from mucus adherant to the biopsy section is perhaps the most sensitive technique to diagnose cryptic infections not found by stool examinations or examination of duodenal contents(16). In the face of negative stool examinations, we have rarely been able to confirm an infection with these methods, perhaps due to the patchy nature of loci of infection or the extreme paucity of parasites in some symptomatic individuals. Two recent reports from India have shown that stools can be positive when aspirates and biopsies are negative(13,18). As duodenal tubal aspiration and intestinal biopsy are bothersome and time-consuming procedures, when giardiasis is strongly suggested by typical epidemiologic exposure and symptomatology, and stool examinations are negative, I prefer to give an empiric course of treatment with the most effective anti-*Giardia* drug, quinacrine (Atabrine). This frequently leads to gratifying improvement in these difficult to diagnose cases. As quinacrine is not known to have other non-specific intestinal effects, such as on enterobacteria, improvement strongly suggests that the drug has acted on a cryptic *Giardia* infection.

### Barium Examination of the Small Bowel

Barium examination of the small bowel may show a characteristic pattern of edema and segmentation(19), but these findings by themselves do not confirm the diagnosis and it is vitually impossible to recognize intestinal parasites for one week or longer after barium use. Many antibiotics, antacids, kaolin products, paregoric, most enema preparations, and oily laxatives can also cause a temporary disappearance from or masking of parasites in the stool. Stool examination of duodenal contents should be deferred for approximately five to ten days after use of these products.

### Serological Testing

Culture techniques are described in other presentations in this Symposium and the described successful efforts in culturing *Giardia* trophozoites may soon lead to the availability of serologic tests for giardiasis.

## TREATMENT

In the United States, three drugs are available for treatment of giardiasis: quinacrine (Atabrine), metronidazole (Flagyl), and furazolidone (Furoxone). A number of other drugs, including tinidazole and nimorazole(20,21), found useful abroad, are not licensed or available in this country and will not be discussed here.

### Quinacrine

Most workers recommend quinacrine as the treatment of choice for giardiasis. We have obtained a cure rate of over 95% with quinacrine in over 100 patients given this drug and most workers report cure rates of at least 90% with it. One remarkable exception to these excellent results is a recent report from London, where *Giardia* was eradicated in only 63% of patients given this drug(12). The dose recommended for those over eight years old is 100 mg three times a day for seven days. For younger children, the dose is 2 mg/kg 3 times daily for seven days. In my experience, though quite effective in younger children, tolerance to quinacrine is poorer in this group. Some intestinal upset, mild headaches or dizziness, and yellow urine commonly occur in all age groups receiving quinacrine. Rarely, vomiting, fever, and severe skin rash occur. We have noted a 1.5% incidence of toxic psychosis in adult patients receiving this drug and patients must be forwarned of this possibility. Yellowing of the skin and sclerae rarely occur in doses used for giardiasis. Quinacrine is contraindicated in those with psoriasis and should not be taken together with alcohol or with primaquine.

### Metronidazole

Metronidazole (Flagyl) is frequently prescribed in this country for giardiasis in an adult dose of 250 mg three times a day for seven days. The recommended dosage for children is 5 mg/kg 3 times daily for 7 to 10 days. In a series of 21 adult cases with established infections, we cured 18, or 85%. In another group of 42 recently returned travelers from the Soviet Union with giardiasis treated with metronidazole 250 mg 3 times daily for 7 days, 27 had one or more followup stool examinations four weeks after completion of treatment and 11 of these were still positive. A similar failure rate of 30% was reported from Sweden in a group of recent returnees from the Soviet Union(21). It would thus appear that metronidazole in the usual recommended dose is less effective in acute than in established infections. This is perhaps related in some way to the excellent absorption of metronidazole. In England, metronidazole in a single 2.0 g dose on three successive days produced a parasitological cure rate of 91%(12), but for reasons to be described, and as the effectiveness and tolerance of these higher doses of the drug are no better than with quinacrine, this regimen cannot be recommended in this country. Although metronidazole in the accepted dose in this country is somewhat less effective than quinacrine, tolerance is generally better. Side effects may include nausea, headache, metallic taste, dizziness, and dark discoloration of the urine. Metronidazole has a well recognized disulfiram-like reaction when taken with alcohol. Metronidazole has been found to be carcinogenic in mice, but not in hamsters, and is mutagenic in bacteria(22), and some controversy has attended its use in non-life-threatening situations. Unfortunately, treatment of giardiasis remains an unapproved indication for metronidazole in this country, and its use in this condition is somewhat debatable. Under these circumstances, it would not appear wise to use this drug in the high daily doses being used in England for giardiasis. Perhaps second thought should be given before it is used in young children and in severely symptomatic pregnant women in whom giardiasis should be treated, at least until it becomes approved for giardiasis.

### Furazolidone

A third drug, which is not widely used here in giardiasis, is the antibiotic furazolidone. It has been found to be effective and rather well-tolerated in a number of studies abroad(20,23). Furazolidone is the only available anti-*Giardia* drug in this country in suspension form as well as in tablets, making it useful in children. In a group of 31 children below age 10 treated with furazolidone, 24 (77%) were considered parasitologically cured by us. Vomiting, diarrhea, nausea, or fever occurred in 10 of 24 of these cases reviewed for side effects, and in three it was necessary to discontinue the drug. There have been other reports of the rare occurrence of hypersensitivity reactions, including hemolysis, hypotension, and urticaria. We recently reported serum sickness in two adults who received tablets of this drug manufactured in Latin America(24). Furazolidone is a monoamine oxidase inhibitor, it can precipatate a disulfiram-like reaction, and it may cause mild reversible intravascular hemolysis in glucose-6-phosphate dehydrogenase deficient individuals. This last situation must be differentiated from the frequently occurring brown color of the urine caused by a metabolite. Also troublesome has been the induction of mammary tumors in rats, which led the Food and Drug Administration to question its use in humans. With satisfactory warnings included in a revised package insert, furazolidone is approved in the United States for treatment of giardiasis. Recommended dosage for adults is 100 mg 4 times daily and for children 6 mg/kg/day divided into 4 doses for 7 days.

### Pregnancy

None of the three available anti-*Giardia* drugs has been proved safe during pregnancy and if used at all during pregnancy, they should be administered only to those women with severe symptoms definitely attributable to giardiasis where benefit is judged to outweigh potential risk. When forced to treat pregnant women, we have chosen quinacrine and have not recognized any deleterious effects on the fetus.

### Asymptomatic Carriers

Although many individuals with giardiasis are asymptomatic when diagnosed and may never become symptomatic, there is the potential for development of intermittent chronic symptoms in some; and particularly with infected children or foodhandlers, there is the possibility of their infecting others(25). I therefore recommend treatment of all infected persons with no contraindication to use of the available drugs, such as pregnancy.

## SOME PROBLEM AREAS

I am certainly convinced that *Giardia lamblia* is a pathogen in many, but not all of those infected, and that it is a leading cause of morbidity. In my practice, I call it "my bread and butter parasite" as it is the most frequently diagnosed cause of illness in my patients. Many others share my opinion. Unfortunately, as with amebiasis, not everyone is yet convinced that it is a definite pathogen and a satisfactory study showing increased morbidity in those with giardiasis over a control population is not yet available.

I have been unable in this presentation to discuss the pathogenesis of *Giardia* in humans, which remains enigmatic. However, much exciting work is being carried out in both humans and in animal models which should help us better understand the pathogenesis. This includes the role of intestinal bacteria and bile salt deconjugation(12); intraepithelial lymphocyte counts(26), and immunoglobulin studies(27), and scanning electron microscope evidence of involvement of the microvillous border and of invasion of the bowel(28). Some of this work will be discussed elsewhere in this Symposium.

An important unanswered problem for me is the patient with typical symptoms and exposure history for giardiasis, in whom we are unable to confirm the presence of parasites by available means, but who responds to specific anti-*Giardia* treatment. It would appear that either our diagnostic methods are not always sufficiently sensitive or that some individuals with extremely light infections may respond very violently. Similar hyperreactivity is seen in the non-immune in other parasitic diseases such as schistosomiasis and malaria.

One excellent drug (quinacrine) and two other quite useful drugs (metronidazole and furazolidone) are available to us in this country, but all have potential problems. Though more effective, quinacrine has the potential to cause serious toxic effects in a small percentage of those taking it, including toxic psychosis, vomiting, fever, and exfoliative dermatitis. Metronidazole, though rather effective and well tolerated remains unlicensed for giardiasis, and as a suspected carcinogen and mutagen, there are potential legal problems should long-term damaging effects eventually be proven. Even now I hesitate to administer it to young children or asymptomatic individuals or in pregnancy. Furazolidone is the least effective available drug; it may cause more hypersensitivity and toxic side effects; and it too has been suspected of having carcinogenic effects. It appears unlikely that other presently available anti-*Giardia* drugs will be approved for use here. A useful future contribution to treatment of giardiasis would be a well controlled comparative study in this country of quinacrine, metronidazole, and furazolidone.

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## Discussion

M. SCHULTZ: I would like to emphasize the point that people with clinical giardiasis are often not diagnosed by their physicians. We found that this was very common early in the development of giardiasis in Americans returning from the Soviet Union.

The story I would like to cite to illustrate this is the case of the wife of an American anatomist. She went with him to Leningrad, and when they returned home she was ill. She had had an ileostomy in place before she took the trip, and when she returned she had copious outpouring of fluid from her ileostomy. She went to see her surgeon who decided to do a resection and when it did not work, he did another resection.

Thereafter, the husband read a story in the New York times that giardiasis was common in American travelers to Leningrad. He bought $1.98 worth of Atabrine (quinacrine) at a drugstore, and without even testing his wife, treated her and cured her promptly.

This is a case of prolonged suffering due to a physician not reaching a correct diagnosis; I have seen many other instances. Those of us who are disciples of this new cult of giardiasis need to preach the gospel about it. It is now the most common intestinal protozoan in America, and an exceedingly common illness in people traveling overseas, as Dr. Wolfe has just pointed out.

M. WOLFE: I see patients all the time who have been previously seen by internists and gastroenterologists. Some of these people have had extensive and expensive workups including X-rays and biopsies, before stool examinations were ever performed. Or because a single stool examination, not performed well in a good laboratory, was negative, their physicians immediately discounted the presence of parasites.

R. RENDTORFF: Dr. Wolfe, I commend you on a very fine presentation, particularly on your description of the epidemiology and clinical features of the disease. I am interested in whether the patients who travel abroad have been on antibiotic therapy, which is a very common form of therapy private physicians render to people who travel to far away places. We have had the news of the value of doxycycline in ordinary traveler's diarrhea disease. Were the patients you discussed on antibiotic therapy which may have influenced this clinical symptomatology?

More importantly, have you used matched controls? I think it is critically important that matched controls be used to determine clinical syndromes, especially ones that have been around for a long time. *Giardia* has been here longer than I have, and I am sure many of the older people here will remember that the incidence of this disease used to be much higher than it is today, and we did not have the kind of fear and clinical worry about the disease that we do today.

I am not saying that I do not believe it is a pathogen under certain circumstances; I think it is. When you mention such things as urticaria and arthritis, it is reminiscent of what happened during the amebiasis scare in the 1940's when amebiasis was accused of everything from falling hair to ingrown toenails. We are getting into a similar situation with *Giardia*. Do you really think arthritis is a phenomenon caused by this parasite?

M. WOLFE: Concerning your point about antibiotics, doctors for my State Department population do not recommend any sort of antibiotic or drug prophylaxis for diarrhea. I have received many calls regarding doxycycline prophylaxis and I steadfastly refuse to recommend or prescribe it to anybody. All of our doctors and nurses overseas have followed this policy.

I think antibiotics do play a role in *Giardia* as does Flagyl (metronidazole). After treatment with Flagyl and antibiotics, patients may experience persistent diarrhea, and sometimes bloating and gas. When we examine their stools with fungus cultures, we sometimes discover a *Candida* overgrowth. This can be an after effect of antibiotics causing these symptoms. I treat people with a possible fungus overgrowth with Mycostatin (nystatin).

I did mention that a good clinical study should be done with matched controls. We have two sets of controls for the 670 cases we have been discussing. These may not represent totally suitable controls since some different questions were asked of the controls. They are matched primarily for epidemiological purposes, not for clinical purposes. You need a prospective study of those who have giardiasis, along with controls, asking them the same questions. However, you will have to differentiate within the controls between people who are symptomatic and totally asymptomatic. We have a group of controls called the "worried well" who think they have parasites. Many of them have parasitophobia and insist on workups every year or every two years when they return from overseas. We are never able to find anything on them, and although sometimes we treat them, they do not get better. They are probably people with irritable bowels.

You have to be careful in selecting controls. In the CDC evaluation of people coming back from the Soviet Union, less than half of the patients were proven to have giardiasis. The other cases were called giardiasis on clinical grounds. There are many people with illnesses that might mimic giardiasis, who if used as controls, will have the same symptoms as your proven *Giardia* cases.

It is a major problem finding suitable controls in a clinical situation where people are coming in after travel because they have problems. Other populations might serve better wherein you can use people who have not been exposed to *Giardia* to the extent people living abroad have, and you can be more certain that they do not have *Giardia*. If you then ask them the same questions as you have asked those in proven *Giardia* cases, you might have a better type of control.

Concerning your point about whether *Giardia* is a pathogen, I can say the symptoms are there. We are seeing a lot of sick people with *Giardia* parasites. We treat them, and the symptoms go away. I agree that some of the bizarre symptoms of arthritis and erythema multiforme are very reminiscent of the great debate that has gone on for years about amebiasis; whether constitutional symptoms such as fatigue and headache and dizziness and other things considerably remote from the bowel are caused by these parasites.

The evidence is that when you treat these people the urticaria goes away, the fatigue goes away, and some of the headache and dizziness goes away. We cannot prove it, but I am willing to accept that many of these symptoms can be caused by both *Giardia* and amebae.

Whether the cause is a toxin or a metabolic breakdown product or some tissue breakdown product, I do not know. When people come in with myalgias and marked prostration, and we find that they have giardiasis or amebiasis, and treat them with what we consider to be very good drugs, many of these people improve. I think there is a very good evidence that these parasites are toxic.

A. CONNELL: Is there any evidence that humans develop acquired immunity in any form? For example, do people who have been treated, and then travel abroad, get reinfected? If they do get reinfected, do they show acute symptomatology or do they develop a chronic type of infection?

In the treatment of cryptic cases, do you see any parasites in the stool and have you followed the stool in these patients with this type of therapy?

M. WOLFE: There seems to be some evidence from the data in Colorado that there might be acquired immunity. In the 670 cases we have been discussing, there are a fair number of repeaters. These are people who have gone abroad for two-year assignments for the most part, or live in Washington and travel abroad on temporary assignments. I have not yet examined these cases carefully enough to see whether these repeated infections give the same symptoms as the initial infection.

It is not often that we see people in the acute stage because they usually have an undetermined duration of infection. They may have been abroad for two years and might have become infected on the first day, the last day, or in between. If they have gone through the typical acute stage that only lasts three to four days, they pass this off as some kind of common diarrhea that people experience.

It is very difficult to work with this population in relation to previous problems. This is especially true if they have been infected for one or two years and there is development of an immunity process. They may well have developed some form of immunity different from that of the traveler who is in and out of the Soviet Union on a one- or two-week trip and then develops the symptoms shortly after he returns to the United States.

There are many intriguing questions which might be answered when we have the proper tools and can measure certain types of immunity, particularly at the tissue level in the small bowel.

A major problem is obtaining enough patients who are willing to subject themselves to duodenal tubing and perhaps multiple intestinal biopsies before and after treatment.

Your other question of so-called cryptic cases concerns people in whom we find no parasitic etiology for their symptoms. However, we are not testing for anaerobes and fungi such as is being done at the Hospital for Tropical Disease in London. If we do not find parasites before treatment of these people, then treat them and they get better, I do not really see an indication to do any follow-up examinations. I do not think it is practical or very worthwhile. Once we treat them and they get better, we release them. If they are still having problems after taking Atabrine (quinacrine) which we find to have a 95% cure rate, the probability of it being giardiasis is not too high. Then we can begin intestinal biopsies, malabsorption studies, and other workup to determine the cause of the diarrhea.

T. NASH: The first major problem I see is the treatment of asymptomatic patients. Is there any general agreement on the role of the asymptomatic cyst passer infecting other groups of people?

The second concerns those patients that present brief abdominal type symptomatology. I wonder if the patient following that actually has pancreatic enzyme elevations and symptomatic giardiasis. How many people have studied or looked at that type of problem in a prospective manner?

M. WOLFE: Certainly regarding children I see every reason to treat them because we have many situations wherein if one child in a family is infected, other siblings may become infected from him. It does not work the other way around as much. An adult who is infected and is asymptomatic and washes his hands, even if he is a food handler, is probably not going to infect other people. Certainly if he is a food handler, though, public health logic calls for him to be treated.

Whether or not symptoms will develop in these asymptomatic people over a long period of time, I cannot say as I do not usually follow them but treat them. It would be worthwhile to follow a group of these people and see whether symptoms develop.

Pancreatic enzymes would be very worthwile to examine. Apparently, the Russians and others in East Europe are doing a great deal of pancreatic enzyme work. The Tropical Disease Bulletin contains many articles about the influence of *Giardia* on liver and pancreatic function. These people are performing some very discreet type of testing that no one has even begun to do here.

D. JURANEK: Just to further emphasize your point about treating asymptomatic patients, especially children, there is at least one study that was done by the Center for Disease Control (Black, et al. Pediatrics, 60:486, 1977). We found that the introduction of a single infected child to a day care center nursery-type situation, rapidly spreads the disease. In this case about 50% of the children developed giardiasis. We compared the prevalance of this infection to socioeconomically matched children of the same age who did not attend day care centers. The prevalence of giardiasis was about one per cent.

M. WOLFE: Giardiasis is very prevalent in institutionalized children, also.

D. JURANEK: We need to better define exactly which type of asymptomatic cyst passer in epidemiologic terms (age, occupation, personal hygiene and sanitation) poses a threat to others and make specific treatment recommendations for that type or group of persons. There are too many asymptomatic cyst passers who pose virtually no public health threat to warrant a blanket statement that everyone be treated.

M. SCHULTZ: The work of Black, et al, was recently confirmed by Keystone in Toronto, in a published paper in the Canadian Medical Association Journal in August. He studied two nurseries in which *Giardia* spread throughout very rapidly, and was significantly pathogenic. He recommended that carriers be treated. He observed that Canadian born children have a much higher rate of giardiasis than children in the same classroom who came from overseas. He speculated, although he did not prove it in any way, that there might be some immunity in the children who had previous infection, and that the children from abroad came into this classroom and were susceptible to infection.

Lastly, on this point concerning immunity in people, Richard Wright, et al *(American Journal Epidem.*, 105:332, 1977) did an epidemiologic study in Colorado which showed that native Coloradoans, who were long-term residents, had lower carrier rates than short-term residents, implying that there might be immunity in long-term residents.

M. WOLFE: As you may know, for years the Russians have been denying that there is a problem with giardiasis in the Soviet Union. We have had very little direct information and some indirect information from translations of Russian literature. However, one notable piece of information in a study of a nursery in Leningrad was that 90% of preschool children attending the nursery were infected with giardiasis. Their literature certainly admits that a problem exists.

W. JAKUBOWSKI: I would like to thank you for an interesting and informative talk, and I think we could probably spend about two and a half days just discussing the symptomatology and the diagnosis and treatment of giardiasis.

I had a close encounter with *Giardia* about 2 years ago, and I had a course of treatment with metronidazole (Flagyl) and also with quinacrine (Atabrine). Five months later the disease spontaneously cured itself. During the course of my infection I had a concurrent yeast infection. In Berlin, New Hampshire, the water concentrates that we examined had large numbers of yeast cells. In your experience in examining stool specimens from infected individuals have you come across any concurrent infections with other organisms?

M. WOLFE: That is an interesting point and it opens up the whole question of what is the possible role of concomitant organisms. Enterobacteria seem to have some association as determined by studies done in England and in India. Some people have postulated that there is some organism, virus, bacteria, or maybe a fungus in the water in Leningrad that makes the disease so much more common in travelers to Leningrad than it seems to be in travelers to other parts of the world.

Speaking specifically of yeast, we see yeast frequently in the stool. It is partially a function of how long an unpreserved stool has stood before being examined, as a small amount of yeast might have multiplied before you examine the specimen. It is possible there may be some inter-relationship with intestinal yeast but I think special attention would have to be paid to this in examining specimens, including fungus cultures of duodenal aspirates, to determine the relation between *Giardia* and the fungus.

Some people post-treatment seem to get a disruption of their normal bowel flora and an overgrowth of yeast, and this can give symptoms not totally typical of *Giardia* but reminiscent of those that occur with both *Giardia* and amebiasis. When you treat for this yeast infection with a non-toxic 7 day course of Mycostatin (nystatin), some of these people do tend to improve. What the interaction is between bacteria and yeast or other organisms with *Giardia* is another interesting aspect that hopefully some of you here will be able to investigate.

# The Possible Use of an Indirect Immunofluorescent Test Using Axenically Grown *Giardia Lamblia* Antigens in Diagnosing Giardiasis

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## ABSTRACT

The possibility of using an indirect immunofluorescent (IIF) test for diagnosing giardiasis is discussed. When we treated *Giardia lamblia* organisms in $10^{-4}$ M 2-mercaptoethanol, before preparing antigens-slides, all nonspecific reactions were eliminated. Seventy-two of 108 sera (66.6%) from patients with symptomatic and asymptomatic giardiasis had titers of 32 or greater, whereas none of 53 normal sera had titers as high as 32. The test-to-test reproducibility was very good, with no difference greater than one fourfold dilution amongG. lamblia antibodies in positive sera were completely absorbed by *G. lamblia* antigens. However, when positive sera were treated with *Entamoeba histolytica, Trichomonas vaginalis,* or *Escherichia coli* the anti-*G. lamblia* titer did not decrease appreciably, which indicates that the IIF reaction is very specific.

In addition to IIF, indirect hemagglutination (IHA), direct agglutination (DA), and double diffusion in agarose gel (GD) were also evaluated. The IIF test was found to be the most useful test and it could be used as an adjunct to stool or duodenal aspirate examination in diagnosing giardiasis.

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*Giardia lamblia,* a protozoan parasite of man, is found all over the world. It is the most frequently diagnosed intestinal parasite in public health laboratories in the U. S., according to an ongoing intestinal parasite survey being conducted by the Center for Disease Control (1). Giardiasis is endemic in the U. S., and an estimated 3 to 7% of the adult population harbor the parasite (2). *G. lamblia* normally resides in the duodenum and upper jejunum and frequently causes acute or chronic diarrheal illness, and sometimes leads to steatorrhea and malabsorption syndrome. In some cases it may even cause lesions in the small intestine (3-5). In other instances the infection may be lost spontaneously after 1 to 4 weeks of patency (6). The reasons for this variation in host susceptibility are not yet fully understood. The high incidence of giardiasis in hypogammaglobulinemic individuals (3); the low infection rate among the permanent residents of Colorado (7) — which is an endemic area, as evidenced by the high infection rates among visiting skiers (8) — and the fact that experimentally infected mice develop prolonged resistance to re-infection with *G. muris* (9) strongly suggest an immunologically mediated phenomenon. Until recently, however, no serological test was available to study anti-*G. lamblia* antibodies in patients' sera. Ridley and Ridley (10) recently described an indirect immunofluorescence (IIF) test using *G. lamblia* cysts as antigen. They found a rough correlation between histological malformation and antibody titer. However, they also reported that finding a reliable source of antigen is always a problem. Another problem that we have had difficulty with is the autofluorescence of the cysts. The successful development of an axenic culture medium by Meyer (11) for the routine cultivation of *G. lamblia* trophozoites solves these problems. Using the axenically cultivated *G. lamblia* trophozoites as antigens, 4 tests were evaluated as potential diagnostic procedures for detecting giardiasis: double diffusion (DD) in agarose gel; direct agglutination; indirect hemagglutination; and IIF. Our preliminary investigation revealed that the IIF test was the most promising. In this report we describe the results of using the IIF test for detecting anti-*G. lamblia* antibodies in the sera of humans.

## MATERIALS AND METHODS

### Antigen Preparation

A strain of *Giardia lamblia* was obtained from Dr. E. A. Meyer. It had been isolated from the duodenal aspirate of a human patient and was grown axenically in Meyer's HSP-1 medium (11), which contains 15 to 20% human serum. Unfortunately, the organisms grown in this medium were found to be unsuitable because they reacted with even normal sera in the IIF test. We then tried to grow the organisms in Meyer's medium containing bovine or rabbit serum instead of human serum and later grew them in Diamond's TP-S-1 medium (12). Actively growing, 3 to 4-day-old *Giardia lamblia* were dislodged from the walls of the 16- X 125-mm screw-capped tubes by immersing the tubes in ice water for 5 to 6 min. The tubes were then vigorously rolled between the palms and centrifuged at 4° C at 250 X g for 10 min in a refrigerated centrifuge (Damon, model CRU-5000 IEC Needham Heights, Mass.).* The supernatant was aspirated, and the sediment from several tubes was pooled and suspended in sterile normal saline in the ratio of 50 ml saline to 0.5 ml of packed cells. The suspensions were then centrifuged as above. The organisms were washed 6 times in this fashion, and the sediment from the final wash was fixed in 1% neutral formalin in normal saline. The parasite suspension was adjusted to obtain 500 to 1000 organisms per low-power (100X) microscopic field. A drop of this antigen suspension was then placed in each of the 12 wells on a slide and allowed to dry at room temperature. Prepared slides were wrapped in tissue paper, labelled, dated, and stored at -70°C until used. Alternately, the pooled organisms from the culture tubes were suspended in normal saline containing $10^{-4}$ M 2-mercaptoethanol (2 ME— saline) and allowed to stand at 37°C for 2 hours. They were then centrifuged as above and washed twice in saline containing 2 ME and a third time in normal saline. The sediment from the final wash was suspended in 1% formalin, and slides were prepared and stored as above.

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**Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.*

Antigens for the DD test were prepared by washing the organisms 6 times in normal saline, freezing the sediment after the last wash at -20°C, sonicating it in a Bronwill sonicator (Biosonic 2A, Bronwill Scientific, Rochester, N.Y.) for 2 min, and centrifuging it at 12,060 X g in a Sorval refrigerated centrifuge (Ivan Sorval, Inc., Norwalk, Conn.) for 30 min. The supernatant was collected, added in 0.5-ml aliquots to a number of vials, and lyophilized.

### Sera

Of the 161 sera tested, 16 were from the Clinic for Tropical and Parasitic Diseases, Toronto, Canada; 19 were from persons with clinical symptoms of the infection during a giardiasis epidemic in New Hampshire; 28 from Cherokee Indian children (collected during 1965-66); 27 from patients seen by private physicians, 6 from CDC personnel returning from trips abroad; 12 from the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; and 53 from normal persons. Of the 53 normal sera, 15 were from CDC personnel and 38 from other normal healthy persons who had no gastrointestinal problems but had donated blood for an arbovirus survey. The sera used in the study were grouped as follows: group 1, 68 sera from clinically symptomatic patients with acute, subacute, or chronic giardiasis who were confirmed to have *G. lamblia* trophozoites and/or cysts in the stools or trophozoites in the duodenal aspirates; group 2, 40 sera from asymptomatic individuals who were excreting *G. lamblia* cysts; group 3, the 53 sera from normal individuals.

### Indirect Immunofluorescence Test

Serial twofold dilutions of each serum (0.05 ml) beginning at 1:2 were prepared in U-type microtitration plates (Linbro Scientific Co., Inc., Hamden, Conn.) using microtitration loops. Phosphate buffered saline (PBS), pH 7.6, was the diluent. The antigen slides were removed from the freezer, washed once in PBS, and allowed to dry. One drop of each dilution of serum was transferred to individual wells in a 12-well slide, and the slides were incubated at 37°C for 30 min in a moist chamber. The slides were then washed three times in PBS at 10 min intervals and allowed to dry. A drop of the conjugate, consisting of goat antihuman globulin labelled with fluorescein isothiocyanate (FITC), CDC F/P 48, protein 40 mg/l, was added to each well of the slide. The conjugate was used at a dilution of 1:500 with a 1:500 Evans' blue counterstain in PBS, pH 7.6. The concentration of the conjugate and the Evans' blue was arrived at by block titration against known positive and negative sera.

The slides were again incubated at 37°C for 30 min in a moist chamber and washed three times in PBS at 10 min intervals. They were then mounted with buffered glycerin, pH 9.0, and covered with a cover slip. The slides were examined with a Leitz Ortholux microscope equipped with a Ploem vertical illuminator, 2 KP 490 exciter filters, and a K 530 barrier filter at 40X magnification. The intensity of fluorescence was graded on a scale of 1+ to 4+ with a 1+ or greater reaction considered positive. In the negative reaction the organisms did not show any fluorescence. Positive control serum was obtained from an individual who had clinical symptoms of giardiasis and was passing *Giardia* trophozoites and cysts in the stool, and negative control serum was obtained from an individual with no history of travel or gastrointestinal disturbance. A PBS control was included in each test run.

The DD test was performed according to Kagan and Norman (13). Three peripheral wells surrounding a central well were cut in the agarose gel. The anti-*G. lamblia* antiserum positive control made in rabbits was placed in the central well and the test materials such as duodenal fluid, *G. lamblia* antigens, and saline were placed in the peripheral wells.

## RESULTS

### Indirect Immunofluorescence (IIF)

Because antigens prepared from organisms grown in HSP-1 medium fluoresced brightly even with normal sera, they were not suitable for the IIF test. Antigens prepared from organisms grown in HSP-1 medium containing either rabbit serum or bovine serum or those grown in TP-S-1 medium fluoresced brightly at the 1:128 or 1:256 dilution of the positive control serum. However, they also fluoresced faintly at the 1:64 dilution of the normal serum. Even though the fluorescence patterns of the positive and negative sera were quantitatively different, we found it difficult to make use of this fact in the absence of a fluorometer. However, the 2 ME-treated organisms were very satisfactory for our purpose, in that most of the normal sera tested did not react at the 1:16 dilution, and most of the sera from symptomatic patients fluoresced brightly at the 1:16 or higher dilution of the serum. The optimal concentration of 2 ME ($1 \times 10^{-4}$M) and the optimal time (2h) for keeping the organisms in 2 ME-saline in order to eliminate all nonspecific reactions were determined by suspending and washing the organisms at different concentrations of 2 ME ($1 \times 10^{-3}$, $1 \times 10^{-4}$, $1 \times 10^{-5}$, $1 \times 10^{-6}$ M) and for different time intervals (60, 90, 120, 150 min) respectively. Fig. 1-5 show the types and degrees to which the positive sera fluoresced at different dilutions relative to those of the negative control at a 1:16 dilution. Neither the negative control nor the diluent control reacted at all at the 1:16 dilution, whereas the 1:16 dilution of the positive control serum fluoresced brightly at 4+ uniformly over the entire surface including the flagella. At higher dilutions the fluorescence was confined to the surface membrane and the intensity was considerably less. The reciprocal of the lowest dilution of the test serum that reacted at 1+ was defined as the titer of that serum. The distribution of IIF titers among the groups of sera tested is shown in Table 1. Fifty-two of 53 (98%) of the sera from normal persons did not react with *G. lamblia* antigens at a titer of 16, and none reacted at a titer of 32. Hence a titer of at least 32 can be considered as diagnostic and indicative of infection. Forty-four out of the 68 test sera (64.7%) from symptomatic patients were positive for *G. lamblia* antibodies at a titer of 32 or greater. Of the sera from patients with asymptomatic giardiasis, 28 of 40 (70%) had titers of 32 or greater. Thus, if 32 is defined as the diagnostic titer, then the test has a sensitivity of 66.6%.

NOTE TO FIGURES 1-5: ***Photomicrograph illustrating the types and degrees of immunofluorescent staining of G. lamblia. Photomicrographic conditions as well as the degree of enlargement and printing time were the same in all cases.***

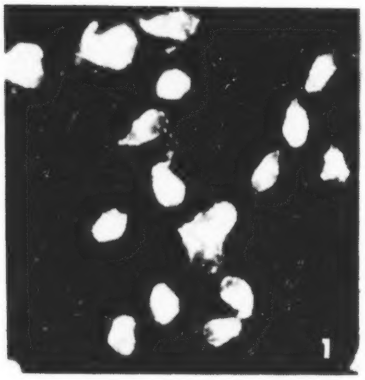

FIG. 1. ***A 4+ reaction at the 1:16 dilution of the positive serum. Note the bright and uniform staining of all the organisms.***

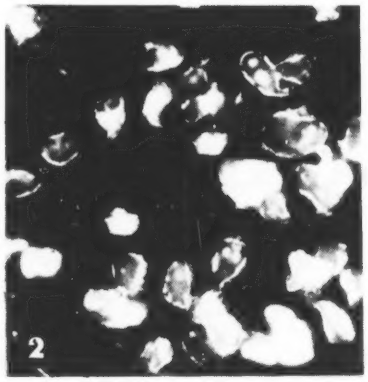

FIG. 2. ***A 3+ reaction at the 1:64 dilution of the positive serum. The staining is confined to the surface membrane in most cases.***

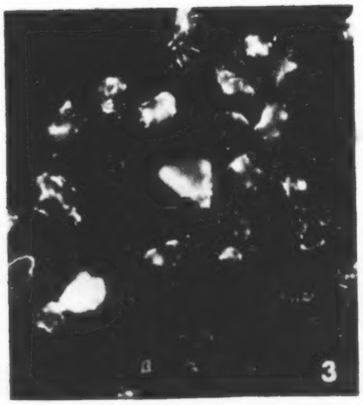

FIG. 3. ***A 2+ reaction at the 1:256 dilution of the positive serum. The staining is confined to the surface membrane, and the brightness is considerably reduced.***

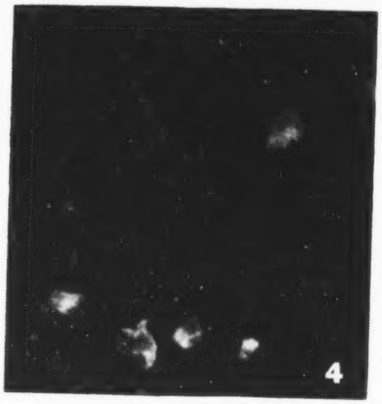

FIG. 4. *A 1+ reaction at the 1:1024 dilution of the positive serum. The staining of the surface membrane is still discernible even though it is dull.*

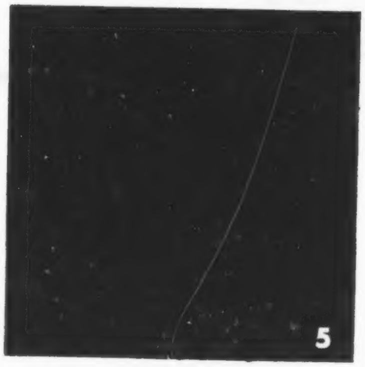

FIG. 5. *Negative reaction at the 1:16 dilution of the negative control.*

In an effort to determine the test-to-test reproducibility and consistency of the test results, we measured the IIF titers on 1 positive and 1 negative serum on 12 different days. The negative serum was consistently nonreactive at the 1:16 dilution. The positive serum had a titer of 256 on 5 occasions, a titer of 128 four times, and a titer of 512 three times (Table 2). The test-to-test reproducibility was very good, with differences in results never ranging higher than 1 fourfold dilution.

One serum with a high titer of 512 and 1 with a low titer of 64 were used to determine the specificity of the test. A 0.5-ml aliquot of each serum was mixed with 0.5 ml packed *G. lamblia* trophozoites, allowed to stand at 37°C for 1h, and stored at 4°C overnight. Samples were then centrifuged at 3000 RPM for 20 min, the supernatants were collected and designated as *Giardia*-absorbed sera, and their IIF titers determined. These 2 sera were nonreactive even at the 1:2 dilution, indicating that all anti-*Giardia* antibodies had been removed. In another experiment IIF titers were determined for 3 sera (2 with titers of 256 and 1 with a titer of 512) after they were similarly absorbed separately with *Entamoeba histolytica* or *Trichomonas vaginalis* trophozoites, or the bacterium, *Escherichia coli.* These absorbed sera

*Table 1. Distribution of IIF titers for sera from selected groups*

| Patient group | Titer 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | Total No. sera |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Symptomatic | 7 | 4 | 4 | 9 | 11 | 11 | 6 | 13 | 1 | 2 | 68 |
| Asymptomatic cyst passers | 5 | 1 | 2 | 4 | 4 | 6 | 0 | 9 | 6 | 3 | 40 |
| Normal | 38 | 8 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 53 |
| Total | 50 | 13 | 12 | 14 | 15 | 17 | 6 | 22 | 7 | 5 | 161 |

*Table 2. Test-to-test reproducibility of IIF titers measured on 12 different days on two sera with* G. Lamblia *antigen.*

| | Titer | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sera tested | <16 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
| Positive control | | — | — | — | 4 | 5 | 3 | — |
| Negative control | 12 | — | — | — | — | — | — | — |

showed no appreciable decrease in IIF titers indicating that they did not contain cross-reacting antibodies.

### Seronegative But Symptomatic Persons

Sera from 24 of 68 patients (35.3%) had titers of 16 or less, i.e., lower than the defined diagnostic titer. These patients were clinically ill with diarrhea and were stool positive for *G. lamblia* trophozoites and/or cysts. The case of a patient who had chronic giardiasis but was consistently seronegative is described below.

*Case of Chronic Giardiasis:* A 29-year-old caucasian female apparently acquired giardiasis in Chile 3 years ago. Since that time, she has suffered episodes of intermittent diarrhea and epigastric pain. She has been treated with atabrine, metronidazole, and furazolidone. She recently completed a course of metronidazole therapy (250 mg 3 times daily for 17 days). She was stool positive for *G. lamblia* before she received this medication and was still positive for *G. lamblia* the day after the treatment ended. One of her samples of the duodenal aspirate was positive for *G. lamblia* trophozoite antigens (Fig. 6) in the DD test. The duodenal sample reacted with the anti-*G. lamblia* antiserum made in rabbits to form 2 precipitin lines, both of which became confluent with 2 of the 7 precipitin lines produced by the homologous antigen-antibody systems indicating antigenic isology. Paired samples of the patient's serum obtained 3 months apart had normal levels of immunoglobulins (Table 3). However, she does not have anti-*Giardia* antibodies.

*Table 3. Immunoglobulin concentration of sera from a patient with symptomatic giardiasis*

| Serum sample | Immunoglobulin (mg%) | | |
|---|---|---|---|
| | IgG | IgA | IgM |
| 1 | 1310 | 120 | 211 |
| 2 | 1350 | 120 | 208 |
| Normal values for 13 yr and older. | (800-1800) | (90-450) | (60-280) |

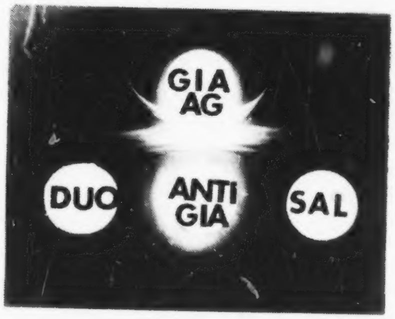

FIG. 6. *Precipitin patterns obtained in agarose gell showing interactions between the antigens of* G. lamblia *(GIA AG) and the giardiasis patient's duodenal sample (DUO) with the rabbit anti-*G. lamblia *(ANTI GIA) serum. Normal saline (SAL) served as the negative control.*

## DISCUSSION

The data presented here indicate the usefulness of the IIF test as a diagnostic tool for detecting antibodies to *G. lamblia* in serum. The IIF test appears to be both specific and reproducible. Absorbing the positive serum with homologous antigens *(G. lamblia)* completely removed the anti-*G. lamblia* antibodies, whereas absorbing the serum with heterologous antigens (e.g., *E. histolytica, T. vaginalis,* and *Escherichia coli)* had no appreciable effect on the anti-*G. lamblia* titer. The test-to-test reproducibility was found to be good, with results never varying more than 1 fourfold dilution. However, the major drawback of the test is its sensitivity; it cannot be used to detect all cases of giardiasis. Only 72 of 108 (66.6%) patients with giardiasis (including both symptomatic and asymptomatic) had diagnostic titers of at least 32. Although no false positives were detected, many false negatives were. A similar problem seems to exist in amoebiasis serology also. Healy et al (14) state that amoebic antibody is not necessarily detected in every infected individual. According to them, "Some people infected with the organism show no antibody to antigens in the IHA test." They think these negative results probably stem from the fact that not all strains of *E. histolytica* are tissue invaders. Neal et al (15) also showed that many asymptomatic individuals who were passing cysts of noninvasive *E. histolytica* in their stools did not have measurable IHA or CF antibodies to these amoebae in their sera. A similar situation may apply to some of our patients who are seronegative but positive for *Giardia lamblia* in stool examinations.

Recently Ridley and Ridley(10) described an IIF test for detecting circulating antibody against *G. lamblia*. They used *G. lamblia* precysts as antigen and obtained positive results for 32 of 36 patients with giardiasis and malabsorption, 0 of 2 patients with giardiasis and no malabsorption, and 10 of 34 patients with malabsorption but with no detectable *G. lamblia* infection. However, they reported having difficulty in obtaining a satisfactory and reliable source of antigen. This problem can be solved by axenically cultivating *G. lamblia* trophozoites. The nonspecific dull fluorescence that we encountered during our initial experiments with axenically cultivated trophozoites was easily eliminated by pretreating the organisms to be placed on slides with 2 ME-saline. The mode of action of 2 ME is not clearly understood, but it probably reacts with the bound serum products and degrades them to unmask antigenic sites and make them available to the anti-*Giardia* antibodies.

Ament and Rubin(3) reported finding no parasites in four of seven patients even after repeated stool examinations. Burke(5) estimated that 10% to 50% of infected individuals do not excrete the parasites in their feces. In this situation, the IIF test would provide a valuable diagnostic tool.

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## Discussion

*Editors' note: This paper was presented by G. R. Healy.*

T. NASH: You didn't mention the specificity of the conjugate you were using. Is it anti-everything?

G. HEALY: Yes, it is anti whole gamma globulin.

T. NASH: What did you take as the end point of your titer in the IFA test?

G. HEALY: We determined a 32 titer as positive. We very seldom had sera that went out much beyond 1024.

T. NASH: It is conceivable that the organisms produced protease of one type or another, that in order to really solidify your absorption experiment, you might want to abosrb sera that has other types of IFA antibodies. I have done similar IFA work with only a handful of patients, not the hundred that you are dealing with, and basically I cannot differentiate between the negative control.

G. HEALY: Were you able to pick up more positives in your symptomatic patients?

T. NASH: I would not be able to state any numbers. I have about 8 sera, but these were all worked up patients that we have had under investigation for malabsorption and at least as far as sera go, using specifically the IgA, IgM and IgG, I was not able to tell very clearly. The IgG seemed to be the antibody of importance; IgM and IgA were not very helpful.

G. HEALY: I have no idea how soon after infection or symptoms these sera were obtained. We do have several individuals at CDC who were symptomatic giardiasis cases. We obtain serum from everybody in the lab every 2 years in case we contract some exotic disease. It is fortunate we had sera from these people, in one case going back to 5 years before infection. Two of them were negative before and after they were infected, and have been serologically negative since. It is very disappointing because we had an opportunity to do some prospective examinations of their samples.

G. HULDT: Your conjugate is polyvalent, but probably reacted mostly against IgG. As this is a small intestine infection with probably very little invasion into the tissues, wouldn't you expect antibodies to be mainly of the secretory IgA class? Have you looked at all for class specificity of your serum antibodies, and have you looked for secretory IgA in secretions, as for instance human milk?

G. HEALY: No, we have not had the opportunity. I think your point is well taken.

G. HULDT: We have tried. We have only been working in this area for a very, very short time and our material is so small. In 15 patients we found remarkably high amounts of IgA circulating and also secretory IgA.

# The Experimental Transmission of *Giardia Lamblia* Among Volunteer Subjects

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## ABSTRACT

Data presented 25 years ago are re-examined in light of present day knowledge of giardiasis. Prisoner volunteers exposed to *Giardia lamblia* by various routes were studied intensively under strictly controlled environmental conditions. In all experiments adequate controls remained negative. Early studies to determine the infective dose showed 5 men failing to become infected after receiving a single cyst given orally in a gelatin capsule. Varying doses from ten to one million cysts produced 100% infection except in a group where only 25 cysts were given to each man. There is evidence of low infectivity in this latter source which produced 6 infections in 20 men exposed. Infections were produced with a single cyst of *Entamoeba coli* and there is no biological reason why single cysts of *Giardia* would not also be infectious. An experiment designed to test for survival of cysts in tap water held at 8°C showed survival for up to 16 days, the longest period tested. One may conclude from this that water is a very efficient vehicle for the transmission of *Giardia* and small numbers of cysts in water could readily produce infections in a susceptible host. Other important factors discussed include that a large percentage of infections clear themselves spontaneously in a relatively short time; that the dose is not related to persistence of infection; that there are possible strain differences in the infectivity of cysts and that stools may have long periods when they are negative in a person who is truly positive. Aside from transient changes toward looser and more frequent stools generally unrecognized by the volunteer, no clinical symptoms could be attributed to these experimental *Giardia* infections.

Between 1950 and 1952 while I was with the Laboratory of Tropical Diseases at NIH, I undertook a series of investigations designed to contribute knowledge concerning poorly understood aspects of host-parasite relationships of human intestinal protozoa. This work was done at the then new Federal Correctional Institution at Seagoville, Texas. These studies were designed to obtain basic information such as the number of parasites necessary to establish the infection, the length of the prepatent period, the persistence of the infections and other aspects of clinical, epidemiological and biological interest concerning protozan intestinal parasites. The original series of papers were published in 1954 (1-3).

It should be stated at the outset that our interest was mainly focused on the problems of amoebiasis. But since we considered it improper at that time to expose prisoners to *Entamoeba histolytica*, the experiments were confined to non-pathogenic amoebae: *Entamoeba coli, Endolimax nana* and *Iodamoeba butschlii*. In these studies we also included *Giardia lamblia*, which at that time was not generally believed to be an invasive pathogenic parasite of man. *Giardia* was thought in the 1950's to cause occasional problems of diarrhea in children but its appearance was so common and, in adults so lacking in clinical symptomatology, that most considered it a non-pathogen. As a result, we felt safe in exposing prisoners to *Giardia.* Fortunately our studies proved this to be correct for we had no evidence of any serious pathogenic effects of the parasites in the volunteers. I will elaborate on this more later. Now a word on how these studies were conducted.

Experiments were conducted in the prison under conditions which allowed for a large degree of control over environmental factors. The degree of this control limited the number of volunteers studied to a maximum of 20 men per group because of physical factors. Information was obtained from 34 *Giardia* infections. This represents the only controlled series of human *G. lamblia* infections produced experimentally.

Participants were prisoners who volunteered after being fully informed of the conditions of the experiments and the risks involved. Very rigid controls were in effect over food and environment during the critical periods of the study. Each subject was quartered individually in a modern brick building and was separated from other inmates. They were fed from a special kitchen and dining room which were under very strict control to prevent transfer of parasites through food and were remote from the main prison cafeteria.

Men selected for the experiments showed no parasites in a total of ten stool specimens collected approximately every other day. They were then studied further under the controlled environmental conditions before being exposed to the parasites. In each feeding experiment some men were chosen randomly to serve as controls and these received no *Giardia.*

Each study group was observed for a period of approximately 6 months, the first 10 weeks of which were under the specially controlled environmental conditions. The remainder of the time, approximately 3.5 months, the volunteers lived under conditions existing in the prison at large. In short, the experiments were conducted in such a manner that the transmission of parasites outside of the experimental means was thought to be very unlikely. This was substantiated by the fact that none of the numerous controls ever acquired the infection.

A medical history was obtained on each volunteer and a complete physical examination was performed at the outset of the experiment and after the experiment was completed. Oral temperatures were recorded daily and weight recorded twice weekly on all subjects during the period of intensive observation. A physician who was available for sick call, administered medicine only when definitely indicated in an attempt to avoid all drugs which might possibly influence the experimental infections.

During the period of intensive observation each entire stool was collected and the weight, color and consistency of each specimen were recorded. Most specimens were examined within an hour after collection but in some cases they were held at 8° C for periods up to 48 hours. All fresh stool specimens were examined by the direct smear technic unstained in saline and stained with D'Antoni's iodine stain. They were also examined by means of a zinc sulphate centrifugal flotation concentration technique. The number of parasites present were counted and the numbers were arbitrarily grouped into 3 categories of parasite density: light, moderate and heavy.

Sources of *Giardia* cysts were donors who were found free of bacterial pathogens and who were followed long enough to be reasonably certain that they harbored only *Giardia*. Cysts were processed in a manner resulting in clean, undamaged parasites. When small numbers of cysts were given they were counted by means of a micromanipulator in a manner in which we were absolutely certain of the numbers up to and including 100 cysts. When cysts were given in doses larger than 100, a suspension of cysts was counted by means of a hemocytometer and appropriate dilutions made to give the estimated desired numbers. All experiments were conducted double blind so that neither subjects nor technicians examining stools knew who belonged to the experimental or control groups.

Various kinds of experiments were eventually undertaken but first the subjects were given accurate known numbers of cysts in gelatin capsules in order to establish the dose of *Giardia* infectious for man. Later experiments were done on the survival of cysts in tap water and experiments were made under simulated natural conditions to study fly transmission.

Table 1 gives the results of the first experiment in which 6 men received *Giardia* with 2 each getting doses of 1, 10 and 100 cysts and 7 men remaining as controls. All of the men receiving between 10 and 100 cysts became infected and neither of the 2 receiving a single cyst nor the 7 controls became infected. The prepatent period is that time in days from when the capsule was swallowed until the parasites were detected by our stated routine methods of stool examination. This ranged here from 9 to 15 days.

In the second experiment 3 men given single cysts failed to become infected. Doses of 10,000, 100,000, 300,000, and 1,000,000 cysts were also given to study the effect of massive doses. As can be seen in Table 2 all these latter men became infected. The shortest prepatent period was 6 days, the longest 11 days. The prepatent period did not seem well correlated to the dose of cysts given. Four men serving as controls remained negative.

In the third experiment 25 cysts were given to each man as shown in Table 3. This and other experiments were designed for purposes not related or pertinent to the study of infective dose but are included because of their bearing on this. This particular experiment concerned the survival of cysts stored overnight in tap water or in saline. Only one of the ten volunteers given cysts became infected and none of the nine controls became infected. The reason for the low rate of infection is not clear particularly in view of the fact that in other studies two out of three men exposed to 100 *Giardia* cysts stored for 16 days in aerated tap water became infected. From prior experience one would expect a dose of 25 cysts to yield a very high infection rate. In this experiment, perhaps the failure may have been due to variations of infectivity of cysts since cysts used in this experiment were from a different source. This of course is conjecture and we never actually tested this hypothesis bearing on the infectivity of the parasite.

*Table 1. Experiment 1. Results of feeding experiments on volunteers exposed to cysts of* Giardia lamblia. *Adapted from reference (1)*

| Group | Volunteer |  |  | No cysts given | Results of stool exam |  | Prepatent Period (days) |
|---|---|---|---|---|---|---|---|
|  | No | Age | Race* |  | Pos. | Neg |  |
| Fed | 18 | 29 | C | 1 | 0 | 50 |  |
|  | 15 | 32 | C |  | 0 | 38 |  |
|  | 19 | 23 | W | 10 | 45 | 49 | 11 |
|  | 26 | 24 | C |  | 3 | 60 | 15 |
|  | 24 | 26 | C | 100 | 20 | 73 | 9 |
|  | 23 | 42 | W |  | 52 | 48 | 10 |
| Controls | 17 | 39 | C | 0 | 0 | 100 |  |
|  | 20 | 20 | W |  | 0 | 56 |  |
|  | 16 | 24 | C |  | 0 | 42 |  |
|  | 21 | 28 | W |  | 0 | 23 |  |
|  | 13 | 23 | M |  | 0 | 64 |  |
|  | 14 | 25 | C |  | 0 | 83 |  |
|  | 25 | 35 | W |  | 0 | 104 |  |

* C=Negro; W=White; M=Mexican

*Table 2. Experiment II. Results of feeding experiments on volunteers exposed to cysts of* Giardia lamblia. *Adapted from reference (1)*

| Group | Volunteer | | | No. cysts given | Results of stool exam | | Prepatent Period (days) |
|---|---|---|---|---|---|---|---|
| | No. | Age | Race* | | Pos. | Neg. | |
| Fed | 27 | 28 | C | 1 | 0 | 53 | |
| | 35 | 46 | C | 1 | 0 | 87 | |
| | 42 | 26 | W | 1 | 0 | 63 | |
| | 29 | 30 | W | 10,000 | 17 | 34 | 10 |
| | 34 | 26 | C | 10,000 | 9 | 72 | 8 |
| | 44 | 26 | W | 10,000 | 30 | 64 | 8 |
| | 4 | 26 | C | 100,000 | 7 | 86 | 8 |
| | 31 | 32 | W | 100,000 | 19 | 94 | 9 |
| | 45 | 39 | W | 100,000 | 24 | 27 | 11 |
| | 33 | 25 | C | 300,000 | 12 | 33 | 7 |
| | 36 | 31 | W | 300,000 | 15 | 83 | 7 |
| | 39 | 25 | C | 300,000 | 9 | 63 | 6 |
| | 40 | 40 | W | 1,000,000 | 6 | 77 | 9 |
| | 41 | 30 | C | 1,000,000 | 4 | 53 | 9 |
| | 28 | 27 | C | 0 | 0 | 69 | |
| | 32 | 37 | C | | 0 | 66 | |
| | 37 | 27 | C | | 0 | 92 | |
| | 43 | 56 | W | | 0 | 68 | |

* C=Negro; W=White; M=Mexican

*Table 3. Experiment III. Results of feeding experiments on volunteers exposed to cysts of* Giardia lamblia.
*Adapted from reference (1)*

| Group | Volunteer |  |  | No. cysts given | Results of stool exam |  | Prepatent Period (days) |
|---|---|---|---|---|---|---|---|
|  | No. | Age | Race* |  | Pos. | Neg. |  |
| Fed Saline | 18 | 29 | C | 25 | 0 | 10 |  |
|  | 20 | 21 | W |  | 0 | 10 |  |
|  | 49 | 30 | C |  | 0 | 14 |  |
|  | 52 | 41 | C |  | 0 | 17 |  |
|  | 54 | 25 | C |  | 0 | 11 |  |
| Tap water | 55 | 36 | W | 25 | 0 | 16 |  |
|  | 58 | 47 | W |  | 0 | 12 |  |
|  | 60 | 31 | W |  | 2 | 12 | 16 |
|  | 61 | 29 | C |  | 0 | 15 |  |
|  | 64 | 25 | W |  | 0 | 18 |  |
| Controls Saline | 46 | 36 | W | 0 | 0 | 8 |  |
|  | 53 | 27 | C |  | 0 | 14 |  |
|  | 57 | 29 | C |  | 0 | 16 |  |
|  | 50 | 27 | W |  | 0 | 15 |  |
| Tap water | 59 | 25 | C | 0 | 0 | 24 |  |
|  | 62 | 26 | C |  | 0 | 9 |  |
|  | 63 | 22 | W |  | 0 | 3 |  |

* C=Negro; W=White; M=Mexican

Experiment 4 was similar to the preceeding one in that men received doses of 25 cysts each as shown in Table 4. One group of 5 men received cysts from donor H; of this group 3 became positive. Another group of 5 men received cysts from donor G; of these 2 became positive. Three controls in this experiment remained negative.

Table 5 sums the results of all the experiments where men were infected with known numbers of *Giardia* cysts. This shows that very small numbers of cysts will produce infections. It seems very likely that in a susceptible host a single cyst would cause infection. This fact is of extreme importance to this conference where consideration is given to the possibility that drinking water containing very small numbers of cysts might be infective to large numbers of individuals. Certainly large doses are not needed to cause infection.

Before going on into some of the biological characteristics of the experimental *Giardia* infections, other of our experiments should be noted. One such experiment dealt with the housefly, *Musca domestica,* as a possible vector of intestinal protozoan cysts. The method of exposing flies to cysts assured that the laboratory reared flies did indeed ingest the cysts into their alimentary tracts. In this particular experiment starved flies were exposed to attractive suspensions of a mixture of *E. coli* and *G. lamblia* and subsequently allowed to attack the food items. Within one hour after exposure to the flies the foods were fed to the men. The foods were not fed directly but were mixed with larger samples which were then fed to all of the men at the same time at their evening meal. Foods involved were various jellos, puddings, custards and milk and exposures were done during 9 of 11 consecutive days. Numbers of flies involved in the experiment varied from 30 to 38 at each exposure. Of the 16 volunteers who partook of the food exposed to the flies, none became infected with *Giardia lamblia* and only a single volunteer subsequently developed an *E. coli* infection. One of the difficulties of this experiment was that the donor F of the *G. lamblia* cysts was the same donor whose dosages of 25 cysts given by capsule produced only a 10% infection rate. We of course were not aware of this at the time and perhaps the experiment would have been successful had we used cysts from another donor. *Giardia* cysts are certainly no larger than *E. coli* cysts and one would have to assume that if the housefly can transmit *E. coli* it could transmit *Giardia.* We concluded however, that the housefly was not a very efficient transmitter of either *E. coli* or *Giardia.*

Of particular importance to this seminar are the water transmission experiments. In 1 experiment a bottle type electrically cooled drinking water dispenser was used along with disposable paper cups in the men's quarters. This dispenser had been placed in the quarters prior to the experiments. Unknown to the volunteers 2000 cysts each of *Entamoeba coli* and of *Giardia* from donor F were placed within the water bottle. The cysts were added to each of 3 bottles on alternate days in January of 1952. There was no control over who drank the water, how much they consumed or exactly when they drank it.

Of 20 men exposed to this water, 4, or 20%, subsequently became infected with *E. coli* and none showed infection with *Giardia.* Again, there is doubt

*Table 4. Experiment IV. Results of feeding experiments on volunteers exposed to cysts of* Giardia lamblia.
*Adapted from reference (1)*

| Group | Volunteer | | | No. cysts given | Results of stool exam | | Prepatent Period (days) |
|---|---|---|---|---|---|---|---|
| | No | Age | Race* | | Pos | Neg | |
| Fed Donor H | 66 | 28 | M | 25 | 0 | 40 | |
| | 39 | 25 | C | | 0 | 25 | |
| | 69 | 31 | M | | 7 | 14 | 14 |
| | 73 | 33 | C | | 6 | 20 | 14 |
| | 74 | 24 | C | | 5 | 7 | 15 |
| Donor G | 75 | 27 | W | 25 | 0 | 21 | |
| | 78 | 26 | C | | 0 | 15 | |
| | 79 | 28 | C | | 7 | 5 | 13 |
| | 80 | 24 | C | | 7 | 4 | 21 |
| | 82 | 25 | W | | 0 | 22 | |
| Controls | 67 | 27 | C | 0 | 0 | 27 | |
| | 72 | 36 | C | | 0 | 60 | |
| | 81 | 37 | C | | 0 | 35 | |

* C=Negro; W=White; M=Mexican

*Table 5. Combined results of all experiments comparing the percentage of men infected with the number of* Giardia lamblia *cysts given. Adapted from reference (1)*

| No. cysts given | No. men exposed | No. men infected | Per cent of men infected |
|---|---|---|---|
| 1 | 5 | 0 | 0 |
| 10 | 2 | 2 | 100 |
| 25 | 20 | 6 | 30 |
| 100 | 2 | 2 | 100 |
| 10,000 | 3 | 3 | 100 |
| 100,000 | 3 | 3 | 100 |
| 300,000 | 3 | 3 | 100 |
| 1,000,000 | 2 | 2 | 100 |
| All doses | 40 | 21 | 52.5 |
| Control | 21 | 0 | 0 |

about the infectivity of the *Giardia* cysts from donor F. One may presume their infectivity was low. A fairly high rate of *E. coli* infections occurred and there is no reason to doubt from a biological basis that the risk of exposure of *Giardia* would not be equal to the risk of exposure to *E. coli*. Either host factors or factors inherent in the parasitic cysts must explain the differences of infectivity rates between these 2 species in this experiment.

In another water experiment using again both *E. coli* and *G. lamblia*, we controlled the variables inherent in the preceeding experiment in a test to show more directly the effect of storage in water upon cysts. One hundred cysts of *Giardia* from donor H were added to 100 ml of aerated tap water contained in a beaker which was covered with aluminum foil and placed in a refrigerator at 8°C. Similar doses of cysts of *E. coli* were also prepared in a like manner. Tests done for residual chlorine in this water proved negative. Two control men received water held under similar situations containing no cysts. Two volunteers were given the water with cysts on the day the beakers were prepared. Three volunteers each received water after 1, 2, 4, 8, and 16 days of storage. The water was stirred in the beaker prior to the men drinking it. On each day that the cysts were given to a particular subject, all of the remaining subjects received plain water. The controls remained negative throughout the experiment. Of the 17 men receiving the *Giardia* cysts all but 6 (64.7%) became infected, while 12 (70.6%) developed *E. coli* infections. The prepatent period ranged from 9 to 22 days and averaged 13.1 days for *Giardia*.

In this particular group of volunteers, we were able to re-expose those who were refractory to infection to cysts of *Giardia*. These men had been followed for a considerable period of time after the original exposures to convince us that they were truly negative. They were then given 100 cysts of *Giardia* in 100 ml. of sterile tap water and 2 of 6 men thus re-exposed did show parasites subsequently.

It would appear that where the dose is sufficient, that water is a very efficient vehicle for the transmission of *Giardia* cysts and these will persist in cool water for periods of at least 16 days. One could presume from the persistent viability of cysts, that they must be infectious for considerably longer than 16 days at least when held at 8°C.

There are many unanswered questions about the biology of *Giardia* that we did not explore in our experiments, which are interesting to speculate upon. It has been mentioned that two of the six men re-exposed to *Giardia* came down with infection. But even more interesting are the four who did not come down with *Giardia*. Would this indicate that these men had a resistance to the organism? It would have been interesting to subject them to still higher doses of cysts. Subsequent epidemiological studies have implied that resistance may be an important factor in human *Giardia* infections.

The biology of experimentally produced *Giardia* infections in man has raised some other pertinent points. First, and most important, is the spontaneous disappearance of the infections. This phenomenon is best presented in graphic form as shown in Fig. 1 and 2, where the solid areas above the line indicate light, moderate, or heavy presence of parasites in stools examined on that day and the shaded areas below the line represent a negative stool. One can argue that the level of parasites' density in the stools is meaningless but the fact that stools became negative and stayed as such is significant.

Among 25 men who were given known numbers of cysts in either the capsule experiments or in the drinking water experiments, 21 (84%), exhibited a spontaneous cure and only 4 showed persisting and apparently chronic infections.

We purged 19 men, who were still available to us, 70 to 90 days after exposure and examined the 2 to 5 stools each deposited on the day of the purge. None showed parasites. One man was screened intermittently over a year's time and never again became positive.

Among those infections showing spontaneous disappearances, the duration of the parasites in the stool ranged from 5 to 41 days, with a mean of 18.7 days and median of 13 days. These results were obtained from the men of the first two experiments, wherein men in Experiment 1 received cysts from one donor; the men in Experiment 2 received cysts from a second donor; and, the men in Experiment 3, on survival of cysts in water, received cysts from a third donor (see Fig. 3 and 4). In this third experiment, the parasites ranged from 25 to 41 days with a mean of 33.9 days and a median of 30 days. The volunteers of the second and third groups differed significantly in the length of duration of their infection. Also, among the men of the last group with the longer period of patency, the pattern of these infections commonly showed a bimodal distribution. This is evident in at least 7 of the 11 men studied.

It should be pointed out that in the apparent persistent infections, there were rather long periods when stools alternated between positive and negative. Volunteers, identified as numbers 102 and 119, established this pattern nicely, wherein periods of negative stools lasted up to 13 days after which time they again became positive.

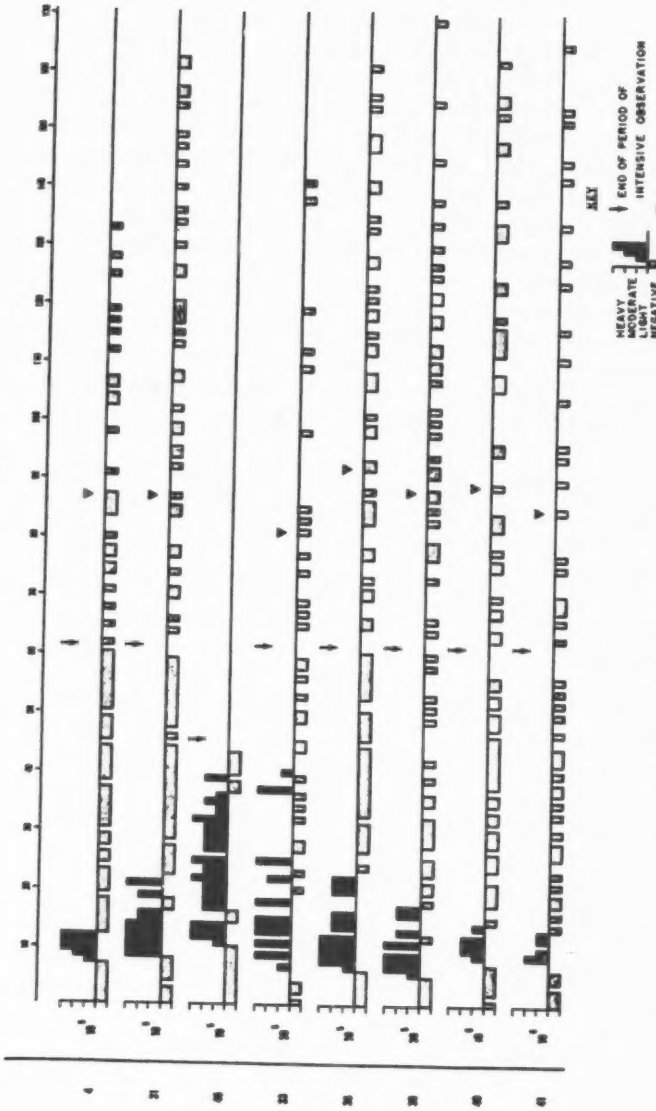

FIG. 1. *Showing the patterns of stools positive or negative for Giardia among volunteers exposed to cysts fed in capsules(1).*

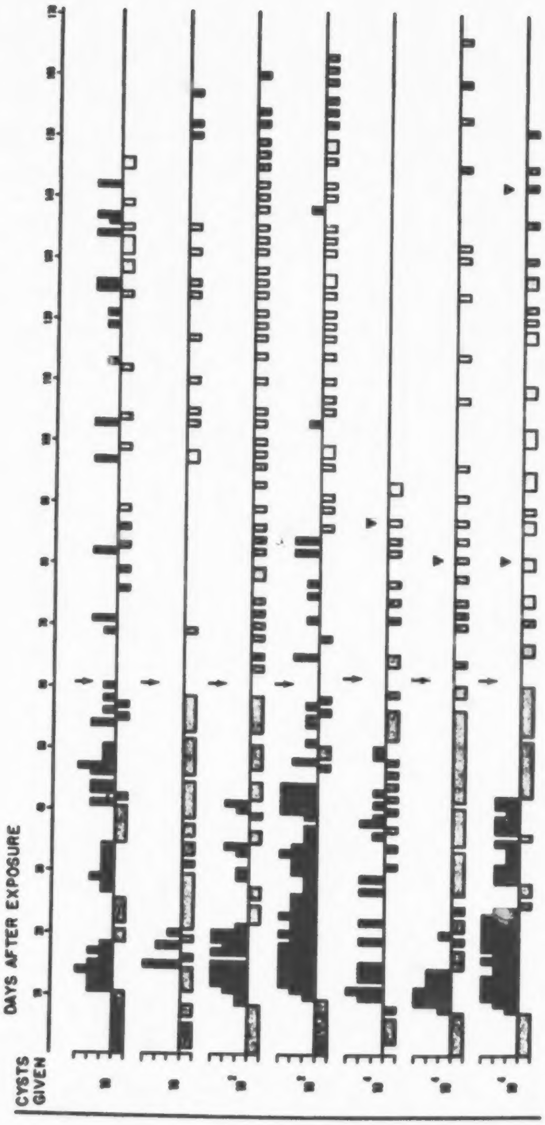

FIG. 2. *Showing the patterns of stools positive or negative for Giardia among volunteers exposed to cysts fed in capsules(1).*

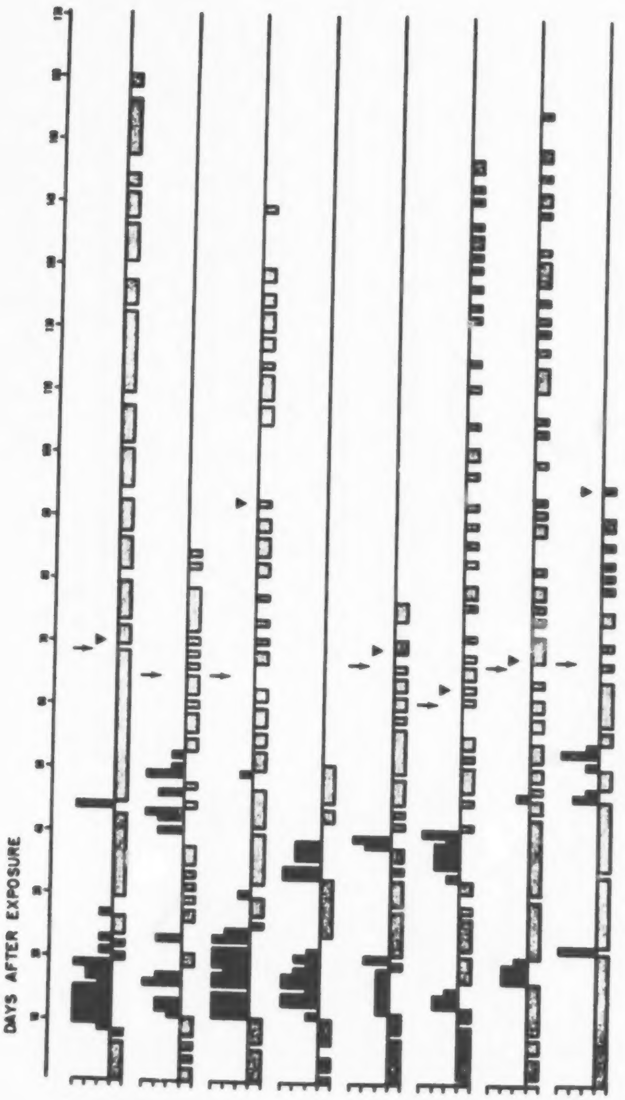

FIG. 3. *Showing the patterns of stools positive or negative for* Giardia *among volunteers who received a dose of 100 cysts in drinking water(2).*

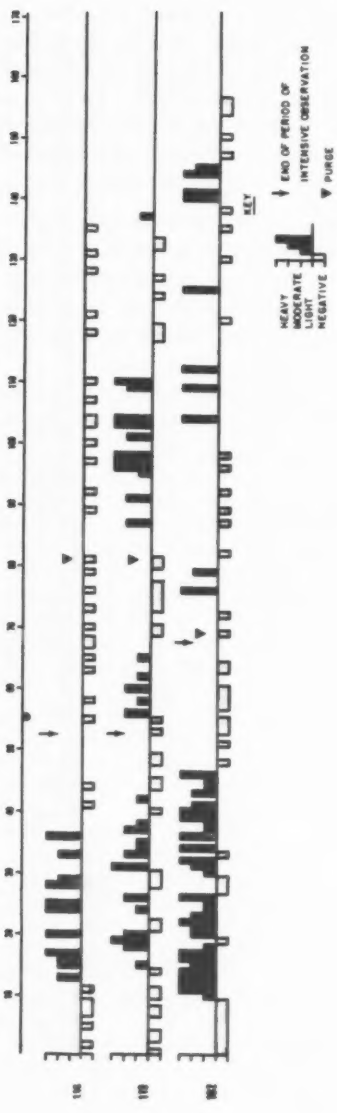

FIG. 4. *Showing the patterns of stools positive or negative for* **Giardia** *among volunteers who received a dose of 100 cysts in drinking water (2).*

Finally, there appears to be little or no association between the dose of cysts given and the pattern of the infection in terms of parasite shedding in the stools. Higher doses of cysts did not lead to permanent infections.

Lessons to be learned so far from these experiments are: a large percentage of infections disappear spontaneously; there may be biological strain differences in the parasite as judged by the different behavior of infections depending upon the donor source; dose is not related to persistence of infection; and, finally, what has already been well recognized in other intestinal protozoa infections, that *Giardia* also may have long periods where stools are negative in a person who is truly positive.

Some of these characteristics of the *Giardia* infections have not been properly considered in subsequent epidemiological and clinical studies done by others. For example, I am not aware of any double blind clinical trial with drugs recommended for giardiasis and in view of the great numbers of our cases that clear themselves without any treatment such trials in my opinion are definitely indicated.

Finally a word in regard to the clinical picture of the *Giardia* infection. This is best presented by reiterating the original from our second paper(1):

> "In a number of the *Giardia* infections a definite change in the stool pattern of the individuals was noticed. This pattern is the correlation of frequency with weight and with the consistency of the stool. It is rather constant for each individual but varies widely between individuals. Thus, in the *Giardia* infections of experiments 1 and 2 there were 4 cases of a marked change in the stool pattern, 5 cases of a moderate to questionable change, and 6 cases where no change was discernible. These changes were always from the normal pattern to one showing more frequent and looser stools and usually stools of greater weight. These changes usually began abruptly with the first appearance of parasites and lasted only 2 to 4 days. In some cases they preceded or followed the first appearance of parasites by a day or two. The change in stool pattern was always transient, lasting at the most but a few days.
>
> Changes in the stool pattern were sometimes very subtle and required actual plotting of the data in graph form to be recognizable. In other instances, such as in volunteer No. 23, these changes were very marked. In this subject the change was noticeable on the day parasites first appeared in the stool and it lasted for 4 days. During this 4-day period 9 stools were passed which averaged 189.7 grams in weight. Of these stools 77.7 per cent were loose, 11.1 per cent were soft, and 11.1 per cent were semiformed. Of a total of 89 stools collected before and after this period of change, the average weight was 127.2 grams and 1.2 per cent were loose, 11.6 per cent soft, 51.2 per cent semiformed, and 36.0 per cent formed. Changes in the stool pattern were rarely noticed by the individual and when they were noticed there was no complaint other than the fact that the movements were looser than usual.
>
> In no instance did any clinical illness or subjective complaint occur among the subjects which could be attributed with certainty or bear close relationship to *Giardia* infections. Gastrointestinal complaints did occur among the infected individuals but these bore no distinct relationship to the *Giardia* infections and also occurred among the non-infected men. One possible exception to this statement was a single case of a man who complained of nausea, vomiting, diarrhea, and abdominal discomfort associated with the first appearance of parasites. However, this subject gave a history of similar episodes prior to his experimental infection and was observed to have such episodes long after the *Giardia* infection had disappeared spontaneously. Other features of his illness deemed it highly improbable that the parasite had any causal relationships to his episodes of gastrointestinal illness."

In closing I want to reiterate my thanks to my research staff and others of the Public Health Service and the Federal Prison Service who made these studies possible. I want also to especially thank the men who volunteered and cooperated so admirably in this study.

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## Discussion

R. OWEN: Since the question exists as to whether it is the parasite or the host that causes those cases which are symptomatic, I want to clarify the source of the cysts that you used. Were any of the people from whom the cysts were isolated people who had been sick?

R. RENDTORFF: They were normal individuals in the prison who volunteered as donors and received benefits for the volunteering. They were all tested, of course, for bacterial pathogens. I am sure they were not passing any pathogens along with the *Giardia.* The difference in the *Giardia* sources was discovered in the experiments and not recognized microscopically or any other way.

R. OWEN: It is conceivable that this was a strain that was not virulent, if such a strain exists, although it may be hard to determine.

R. RENDTORFF: We used many different donors in the experiments.

R. OWEN: There is an increasing awareness that *Giardia* can be transmitted as a venereal disease, as can any of the other enteric pathogens. Many studies indicate that there is a high degree of venereal transmission within prison populations. Recrudescence of stool excretion in most prison populations would be difficult to differentiate from venereal transmission or retransmission of *Giardia.* The exception would be those prisoners who were in solitary cells.

R. RENDTORFF: Are you referring to the variations in negative and positive findings of parasites?

R. OWEN: Yes.

R. RENDTORFF: This is true of all the other parasites we used. It has been experimentally known long before our work with monkeys using *Entamoeba coli* and other intestinal protozoa. It has no relationship.

The experiments we have been discussing were performed in a model prison institution, without bars, within the federal prison system. I do not know if homosexuality was prevalent in that prison, as it is in the crowded prisons today. Sexual mores were different 30 years ago. This activity was at a minimum and unknown to me, and I really do not believe it ever occurred in our experimental group. They were under very close observation and were intent on achieving their meritorious good time for volunteering for the experiments.

M. WOLFE: These were very unique studies and have become historical in terms of volunteer infections of *Giardia.* You referred to use of clean, undamaged cysts and their survivability in stools kept for 24 to 48 hours before cysts were separated. Were you able to test for viability of these cysts?

R. RENDTORFF: No. To my knowledge, there is no test for viability of cysts that can be performed without killing the cyst.

M. WOLFE: I think there may be some vital stain tests which can be performed to test viability.

R. RENDTORFF: I do not think you would want to use those cysts when they are to be given to men. I really do not understand the essence of your question.

M. WOLFE: The essence of my question is that maybe these cysts were not all alive when you gave them to the men. Maybe that is why they had self-limited infections with short-lived durations.

R. RENDTORFF: They were not all self-limited. Some lasted for long periods of time.

M. WOLFE: Perhaps it took only one viable cyst out of twenty-five, or as many as a hundred viable cysts in one patient to give him a persisting infection. Others may not have taken in any viable cysts because of the circumstances under which your experiments were performed.

R. RENDTORFF: I think there is a biological difference in the donors which can determine low and high infectivity of cysts. This particular donor, as a source, was apparently low in infectivity. However, in the others, I do not see how this could possibly be the case. The cysts were of high viability and infectivity because very low numbers of them produced infections that lasted very long times, and the numbers of parasites found were enormous. This was not just a low grade infection.

As few as 10 cysts produced infection; that is a very small number. In our *E. coli* and other tests, 1 cyst produced good, solid infections. I cannot see the question of viability in regard to this except in the case of that one donor where there is some question about his cysts. I believe that strain differences cause different infection rates.

W. JAKUBOWSKI: As a result of your experiments with the prisoners, along with other information you have obtained, is there any question in your mind regarding the pathogenicity of the *Giardia* organism?

R. RENDTORFF: The results of our prison experiments convince me that the parasite that these men had was non-pathogenic. That does not mean I do not think the parasite could cause disease. I am right now working on a very fascinating disease, Legionnaires' disease, which infects only certain individuals from groups of people. Why only certain individuals are infected with Legionnaires' disease or become infected by *Giardia* while others do not, is unknown to me.

I think the infectivity rate is a host factor we have yet to define. We know that the host factor is true for almost all diseases. There are some diseases that attack children; others attack adults. The evidence of immunity is an extremely interesting topic which indicates that this may be an extremely important factor in the spread of giardiasis. It is a topic that we thought of years ago; however, we did not refer to it in any of our publications.

We were convinced years ago that *Giardia* is spread very rapidly among people. The evidence today, based on outbreaks of *Giardia* in orphanages and places where young children are crowded together, indicates that *Giardia* spreads like some viruses and other diseases found in young children. It is not surprising that *Giardia* is transmitted from person to person. It is important to understand this relation of the transmission of *Giardia* in waterborne outbreaks among human populations. A great deal of study needs to be performed in this area.

W. JAKUBOWSKI: Independent of host differences, it is also being considered that there might be virulence factors in different strains of the organism. Would you care to comment on the possibility that we might be dealing with more virulent strains of the organism resulting in an increase in the number of outbreaks of the disease?

R. RENDTORFF: I do not know why a virulent strain would persist in water rather than a non-virulent one. Virulence is a factor of the parasite, not the host. The evidence for virulence, in our experiments, is that cysts from one donor did not cause as great a number of infections as we expected. Certain strains of *E. histolytica* vary in virulence remarkably as evidenced by the severe outbreak at the Chicago World's Fair. Other strains apparently are less infective, and there is no reason to believe that *Giardia* may not have the same variance.

# Giardiasis in the Mouse: Clues to Host Immune Mechanisms

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## ABSTRACT

The role of host immunity in human giardiasis is poorly understood. Prospective studies in a murine model of this infection have been facilitated by the development of quantification techniques. Following primary infection, mice are resistant to reinfection. Protective immunity is not achieved in congenitally athymic mice. Protection against infection is transferred passively from immune mothers to suckling mice via breast milk and is associated with specific anti-*Giardia* IgA. Immune lactating females lose their intestinal resistance to *Giardia* but regain it after weaning. Studies in mice may lead to appropriate investigations in human giardiasis designed to achieve immunoprophylaxis for this infection.

## OBSERVATIONS WHICH SUGGEST IMMUNITY IN HUMAN GIARDIASIS

The influence of host immunity on the clinical course of giardiasis remains undefined. Giardiasis may range from a short, clinically innocuous infection to a prolonged, debilitating illness(1). It may resist repeated chemotherapeutic efforts to eradicate it, or it may be short and spontaneously self-limited (2). There remains no explanation for the striking variability of clinical illness in this infection. Nevertheless, protective immunity is suggested by the lower prevalence of cysts in stools of long-term residents in areas of high endemicity such as Aspen, compared to brief visitors to these locations(3). Epidemic illness in Aspen during an outbreak was present in 11% of vacationing skiers(4) compared to 2% in permanent residents(5) studied in a separate survey.

One of the observations in humans which suggests a role for the immune response in host resistance to *Giardia* is the association of giardiasis with immunodeficiency. As many as 80% of persons with variable immune deficiency syndrome and diarrhea may have *Giardia* in the stool(6). In this setting, giardiasis is usually accompanied by marked morphological changes in jejunal histology.

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Preliminary studies into the demonstration of specific immunity to giardiasis have been inconclusive. Serum immunoglobulin deficiency was not observed in 70 otherwise healthy travelers with giardiasis(7). In another report of preliminary studies, circulating antibodies of the IgG class were detected by indirect immunofluorescent techniques. This work was apparently handicapped by difficulties in obtaining adequate purified specimens of cysts or trophozoites for antigen source(8). While one group reported a reduction of duodenal IgA, with normal circulating IgA, in 10 subjects who had recovered from giardiasis(9), their collection and assay methods were later criticized(10) and the observations have not been confirmed by subsequent investigators(11).

## PROSPECTIVE STUDIES IN MURINE GIARDIASIS

### Basic Model

**Why the Mouse?**

The mouse was chosen for prospective studies of giardiasis(12,13) for practical reasons. Firstly, there are well-defined inbred murine strains on which to perform immunological studies. Secondly, many basic immunological investigations have been performed using the mouse. Thirdly, considerable immunological technology on the mouse is available on which to base investigations of its immune response to this protozoan. Finally, the mouse is small, easily handled, and economical.

**Quantification Techniques**

Methods of quantification of infection were developed to measure intensity and duration of infection. Quantification is not generally considered a meaningful measurement in protozoan infection as it is in helminth infections(14). However, the characteristics of giardiasis which we have observed in mice makes quantification both appropriate and, indeed, meaningful.

Isolation and quantification of cysts from the stool employs techniques developed in this laboratory based on a method originally employed for ultrastructural studies(15). Trophozoites are counted by flushing free trophozoites from the entire small intestine by irrigation with a known volume of physiologic saline followed by enumeration in a hemacytometer counting chamber. Non-fasted mice are isolated in individual cages for two hours. All stools obtained during this period are broken up in 3 ml of tap water and isolated on 2.5 ml of 1 M sucrose (specific gravity 1.11) in a 75x12 mm plastic tube and centrifuged at 400 X g for 15 minutes at 20° C. Cysts are removed from the water-sucrose interface by careful aspiration, washed by resuspension in 4 ml of physiologic saline and centrifuged at 600 X g for 10 min. The cysts are resuspended in saline and counted in the hemacytometer chamber. This technique yields a recovery rate of 65% of cysts in the original specimen consistently over a range of 10,000 to 100,000 cysts(12).

***Giardia muris* Adapted from the Hamster**

The *Giardia* strain employed in these studies was originally isolated from naturally infected golden hamsters in the Case Western Reserve School of

Medicine Animal Facility. The strain was recovered by direct peroral inoculation of *Giardia*-infected stool into non-immune CF-1 mice. Subsequent passage of this strain in stool extracts was carried out from mouse to mouse. This strain has now been passaged over 50 times in subsequent generations of CF-1 mice. Since its adaptation to mice in 1975, the strain has been distributed to laboratories for studies elsewhere in the United States and Australia. Subsequent attempts to adapt other hamster *Giardia* strains to mice have been uniformly unsuccessful in our laboratory (D.P. Stevens and J. Houser, unpublished) raising questions of why this particular isolate passes readily. Ultrastructural studies, however, have demonstrated unique morphologic aspects of this strain (P. Nemanic, R. Owen, and D. P. Stevens, unpublished) and may shed light on criteria for strain specificity in various *Giardia* species.

### Susceptible Murine Hosts

Numerous mouse strains have been employed, but the bulk of the studies to be described were performed in female CF-1 strain albino mice (Carworth Farms Division, Charles River, Portage, Michigan). Susceptible inbred strains have included Ajax, BALB/c, nude and others (13). Careful selection of supplier and breeding stock is important since many colonies are naturally infected with *Giardia*. CF-1 mice obtained from Charles River and inbred strains obtained from Jackson Laboratories, Bar Harbor, Maine, have been uniformly free of previous *Giardia* infection.

CF-1 mice are obtained at 12 to 14 g weight (5 to 6 weeks of age) and are immediately screened for the presence of cysts in stools. Freedom from infection at this age insures freedom from previous infection, since studies in newborn animals indicate that susceptible suckling animals inoculated as early as 2 days of age will remain infected when tested at 6 weeks of age (D.P. Stevens, unpublished). Freedom from previous infection is also assured by demonstration of susceptibility to infection of randomly selected animals from each shipment.

### Murine Model: General Characterization

When simultaneous measurements are made of trophozoites in small bowel and cyst excretion in stool, the curves which describe these counts over time are parallel (13). Therefore, the level of cyst excretion in the mouse model may be interpreted to reflect intensity of trophozoite burden in the small intestine. This observation, which reflects a difference from human infection where numerous trophozoites may be found in the small bowel when cysts are undetectable in stool lends itself to longitudinal studies of the same mice. Nevertheless, while resolution of infection in this model occurs 6 to 8 weeks after inoculation when the sucrose flotation technique is used for cyst quantification, absolute elimination of infection is probably not achieved. Animals in our laboratory will excrete small numbers of cysts on isolated occasions long after clearance of infection. Such animals, however, are resistant to the development of measurable infection after reinoculation. Moreover, previously infected animals in which cysts are undetectable in stools can still be found to harbor small numbers of trophozoites in the intestine if the entire small intestine is flushed with large volumes of saline and concentration techniques are then employed (D.P. Stevens and D.M. Frank, unpublished).

The studies described using this model were conducted using oral inocula in the range of 500 to 1,000 cysts. The Infectious Dose$_{50}$ (ID$_{50}$) in our laboratory for this strain is, however, between 25 and 50 cysts (D.P. Stevens and D.M. Frank, unpublished).

### Comparisons of Murine and Human Giardiasis

An important question remains: does infection in the mouse result in disease analogous to that observed in man? Jejunal morphology, while less strikingly altered than that seen in severe giardiasis in man, is rendered abnormal by *Giardia* infection in the mouse as determined by villus:crypt ratios (12,13). The villus:crypt ratio is 3.1±0.1 in jejunum of uninfected control mice in biopsies obtained 10 cm from the gastroduodenal junction. The same measurement made at the peak of infection following inoculation of 10,000 cysts shows a villus:crypt ratio of 2.1 which returns to normal after resolution of the infection. Furthermore, weight gain is retarded in infected animals compared to control animals. Mean weight gain for 16 g mice was 8.25±0.4 g in control animals while that for animals inoculated with 100, 1,000 and 10,000 cysts was 6.88±0.3, 6.13±0.7, and 5.00±0.5 g respectively over a 28 day period. These figures are significantly different from uninfected animals for all groups ($p<0.02$). Although *G. muris* infection in CF-1 mice results in moderate jejunal morphologic changes and retarded weight gain, infection in mice appears clinically milder than the most severe form observed in man and appears to be more uniform. Nevertheless, the changes are sufficiently similar to suggest that the study of this model will be relevant to an understanding of the interaction of host and parasite in humans.

## IMMUNE STUDIES IN THE MOUSE

### Demonstration of Immunity

We established in CF-1 mice that resistance to reinfection developed after resolution of the primary *Giardia* infection(16). Sixty-four previously uninfected mice were divided into two groups, the first of which was inoculated on day 0 with 1,000 *G. muris* cysts orally while the other group served as uninoculated controls. At 6, 12 and 18 weeks animals were selected by lot from each group and challenged with 1,000 *G. muris* cysts orally. Animals which previously had been infected demonstrated resistance to reinfection at each of the 3 time periods. The control animals went through the usual expected pattern of peak excretion at 7 to 14 days followed by gradual resolution at 6 to 8 weeks as repeatedly seen in primary infections in other mice. The presence of resistance for up to 18 weeks in these animals and up to 24 weeks in animals used in other experiments, as well as clearance of infection following primary infection in all animals, indicates that resistance to murine giardiasis is acquired after primary infection and probably reflects immunity against this protozoan.

**Immunity is Thymus-Dependent**

All inbred strains of mice do not resist reinfection uniformly. We have observed this resistance in Ajax and BALB/c strains and the pattern of infection is similar in these inbred strains to that seen in CF-1 animals. When congenitally athymic (nude) mice were tested for resistance to reinfection, however, typical resistance did not develop(17). Groups of 14 nude mice and 14 heterozygous thymus-intact animals were inoculated orally with either 1,000 *G. muris* cysts, or in the case of controls, a similarly processed but *G. muris*-free stool extract. Cyst excretion was measured twice weekly. Cyst excretion in nude mice rose to peak levels at day 18 and persisted at high levels until day 46 and then gradually returned to reduced but persistent levels. In contrast, maximum cyst excretion occurred in thymus-intact controls on days 7 to 14 and returned to undetectable levels by 42 days after inoculation, a pattern identical to that seen in other immunologically-intact strains.

Uninoculated animals in both groups remained free of *Giardia* infection throughout the 130 days duration of the experiment. Wasting or fatal disease was not observed in the athymic animals as has been described by others in naturally infected(18) or prospectively infected nude mice(19). The explanation for the differences observed in clinical illness in athymic mice in this laboratory and those of others, may be associated with differences in nude strains or may be a reflection of synergistic infection by other infectious agents in the animals that became ill. It is recognized that nude mice are extremely susceptible to a wide variety of infections(18). We are confident that additional pathogens were not introduced in our inocula since our control animals were inoculated with identically processed but *Giardia*-free stool extracts and they remained free of illness as reflected by their maintenance of weight and absence of mortality.

At least one thymus-intact inbred strain has been studied in which *Giardia* infection does not spontaneously resolve(19). This suggests that explanations other than thymus-dependency may be involved in the development of immunity against *Giardia* and may include the congenital absence of an immune response to *Giardia* antigens. Moreover, preliminary data derived from studies of $F_1$ hybrids of resistant and non-resistant strains suggests that the ability to develop resistance to *Giardia* in the mouse may be a dominant genetic trait(19).

The precise role which the thymus-dependent immune system plays in resistance to *Giardia* remains speculative. Little morphologic evidence is available that actual cellular immune mechanisms are active in the resolution of *Giardia* infection. Increased numbers of lymphocytes are observed in the lamina propria of *Giardia*-infected mice(20). Demonstration of juxtaposition of intraluminal lymphocytes and *Giardia* trophozoites in the jejunum of infected mice by means of scanning electron microscopic techniques (R. Owen, P. Nemanic, and D.P. Stevens, submitted for publication), while provocative, does not clarify the mechanism of the lymphocyte-trophozoite interaction.

## A Role for Secretory IgA

Because the elaboration of secretory IgA is a thymus-dependent process, it is reasonable to suspect that this local humoral immune mechanism may be active in the immune response to *Giardia.* To approach this hypothesis, studies were performed to test the ability of immune mothers to protect *Giardia*-inoculated offspring from infection(20). Accordingly, female CF-1 mice were divided into *Giardia*-inoculated and uninoculated groups. All were observed weekly by quantification of cyst excretion to document the establishment and resolution of infection in the experimental group and the absence of infection in the control group. After resolution of primary infection, resistance to reinfection was demonstrated in the immune group by re-challenge with 1,000 cysts orally. The immune and non-immune groups were then bred to *Giardia*-free CF-1 males. Within 1 week of delivery of the resulting litters, the offspring of both groups were challenged orally with 500 *G. muris* cysts each. Ten days later the offspring were sacrificed and the intestine of each suckling mouse flushed with 5 ml of physiologic saline. The trophozoites obtained from each animal were quantified in a hemacytometer counting chamber. Infection occurred in all 27 mice suckled to non-immune mothers while it was absent in 30 of 31 animals suckled to immune mothers.

The possibility remained that protection might be transferred transplacentally via IgG. Therefore similar experiments were performed whereby litters from immune mothers were inoculated with 500 *G. muris* cysts and then fostered on non-immune wet nurses. Similarly, offspring from non-immune mothers were fostered on immune wet nurses. When trophozoites were examined 10 days after inoculation in these offspring, infection was undetectable in all animals suckled to immune mothers while it was present in 12 of 14 animals suckled to non-immune animals. That protection was absent when animals were no longer suckling, was established by demonstration of infection after inoculation of weaned animals regardless of the immune status of the animal on which they were previously breast-fed. Preliminary studies using indirect immunofluorescence studies which employed purified *G. muris* trophozoites as antigen indicate that such protection is associated with the presence of specific anti-*Giardia* IgA in breast milk of lactating immune CF-1 females (J. Andrews and D.P. Stevens, unpublished). These data demonstrate that in the mouse resistance to *Giardia* is passively transferred in maternal milk and, moreover, suggest that the secretory IgA system may be involved in host immunity which is developed against this organism.

## Loss of Maternal Intestinal Resistance During Lactation

Previous studies have emphasized the role of the gut-associated lymphoid tissue which provides the lymphoid population that homes to the mammary glands and proliferates to lead to secretion of IgA in breast milk(22). We studied the effect of lactation on maternal intestinal resistance to *Giardia* at the time of lactation(21). Immune female CF-1 mice that had undergone primary infection 5 months previously were bred to *Giardia*-free CF-1 males, and maternal cyst excretion was measured twice weekly. While stool cyst excretion had remained absent for the 14 weeks following resolution of primary infection, it spontaneously commenced at 5 to 8 days before the onset of lactation. Maternal cyst excretion in these "immune" mothers rose to levels comparable to those seen during primary infection and persisted as long as lactation persisted. Immediately upon cessation of lactation by removal from the mothers of the suckling litters, whether at 5, 10, 15 or 21 days after birth, maternal fecal cyst excretion returned to undetectable levels in the mothers within 7 days. These observations suggest that maternal intestinal resistance to *Giardia* in immune females is lost during lactation. This may be secondary to migration of gut-associated lymphoid tissue to mammary sites, although mechanisms other than trafficking of these lymphocyte populations cannot be ruled out by these data.

## FUTURE PROSPECTS

Current studies are underway in this and other laboratories using the murine model of giardiasis to better understand *Giardia* antigens and the detailed mechanisms of the immune response which they elicit. Because the natural history of this protozoan infection and the host immune response are so poorly defined in the human, the use of an animal model lends itself to a better understanding of these aspects of this infection. Fortuitously, the precise immune status of the host can be defined in prospective observations in the mouse. It is therefore hoped that such studies will lead to experiments and observations in human subjects which will be meaningful and lead ultimately to the practical goal of immunoprophylaxis in giardiasis.

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## Discussion

S. ERLANDSEN: Did you use the *Giardia muris* or *Giardia lamblia* species in your experiments?

D. STEVENS: We are calling the strain which came from a hamster *G. muris*. We subsequently looked at cysts from other hamsters, but we were unsuccessful in infecting mice with these strains. I am uncertain if this is a characteristic of this particular strain from this particular hamster.

Some of the morphologic observations that R. Owen has made on the strain which he will discuss tomorrow night may be relevant, such as the presence of the endosymbiont in some of our strains.

SPEAKER: The data on the offspring were extremely interesting. The intestine is extremely different up through about three or four weeks of age, and so you are seeing something which is perhaps a little unique in some cases because that intestine is still capable of absorbing maternal immunoglobulins.

Did you look at trophozoites in the intestine, since we are looking at cyst production here, or cyst shedding? Were they gone at the same time? Would this indicate perhaps a control over cyst production versus trophozoite numbers or perhaps both?

D. STEVENS: When adults stop shedding cysts and the cysts are undetectable in a two-hour stool collection, looking for trophozoites in the intestine yields about a 65 per cent recovery rate. Consequently, the absence of cyst excretion does not represent total immunity. Moreover, in maternal infections, there were spontaneous infections which developed presumably from trophozoites, present in the intestine. As in most protozoan infections, the immunity, if it occurs—and we think it does—is certainly not absolute in this animal.

T. NASH: Is this relevant to the immunodeficient patients who are gammaglobulinemic? Is the abnormality the same or different?

D. STEVENS: The nudes obviously have a different congenitial abnormality; however, some similarities may exist. But truly, it is not the same disease. The nudes have a different disease than the immunodeficient patients that we see in that the former have a cellular immune deficiency and the latter a humoral deficiency.

T. NASH: I am not sure that the G cell dependent has been properly examined.

D. STEVENS: We did not set out to establish a model of immunodeficiency. We were looking at the question of thymus dependency of immunity in giardiasis.

G. HULDT: Did you put the thymus back into your nudes and measure that effect on your production?

D. STEVENS: No. Dr. Roberts-Thomson has restored nudes intravenously using thymocytes and has shown partial restoration of resistance.

G. HULDT: Did you just look for *Giardia* in the mice or did you look for antibodies?

D. STEVENS: The results of antibody testing are so preliminary that we would be reluctant to discuss them in this forum; we may be better prepared to discuss it later. Not only does the median body of the *Giardia muris* differ from that of *lamblia* and other species, but *Giardia muris* is the one strain in nature that Meyer or others have been unable to cultivate axenically, thus making purified antigen very difficult to obtain. However, using some work J. Andrews in our lab has done, we are now at the stage where we feel we may be dealing with some purified antigen preparations, and in immunofluorescent studies, we are pursuing experiments in the mouse similar to those discussed with G. Healy this morning.

# SESSION III - EPIDEMIOLOGY

*Chairman - Myron G. Schultz*
*Center For Disease Control, Atlanta, Georgia*

**The Presence and Absence of *Giardia lamblia* in Studies on Parasite Prevalence in the U.S.A.**
**G. R. Healy**

**Animal Reservoirs and Cross-Species Transmission of *Giardia***
**R. B. Davies, C. P. Hibler**

**Waterborne Outbreaks of Giardiasis**
**G. F. Craun**

**Waterborne Giardiasis**
**D. Juranek**

**Water Supply Problems Associated with a Waterborne Outbreak of Giardiasis**
**E. C. Lippy**

**An Outbreak of Gastroenteritis Associated with *Giardia lamblia***
**L. Veazie, I. Brownlee and H. J. Sears**

# The Presence and Absence of *Giardia lamblia* in Studies on Parasite Prevalence in the U.S.A.

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## ABSTRACT

*Giardia lamblia* is currently agreed by most people to be a pathogenic protozoan parasite of the upper digestive tract. In the past when stool surveys were done, the organism was treated merely as another commensal gut parasite. Different prevalence rates recorded in various stool surveys probably resulted from true differences between various populations but also from a number of other factors: 1) differences in stool examination techniques utilized, 2) the use of preservatives in specimens, 3) the number of stools examined, and 4) the inherent variability with which *G. lamblia* is excreted. A diagnosis of *G. lamblia* infection is still largely dependent upon recognizable morphologic features of the organism.

If we are to do a better job of determining the infection in an individual patient or the number of people infected in a waterborne outbreak, stool techniques must be developed which will detect the organism's presence whether or not its morphologic profile is intact.

*Giardia lamblia* has had a variable reputation as a parasite of the upper digestive tract of man. An analysis of stool parasite surveys conducted over the past 55 to 60 years in the United States indicates that of the approximately 100 papers published, relatively few attempted to assess the prevalence of *G. lamblia* itself (1,2). The organism has had to occupy second place to *Entamoeba histolytica*, which was long believed by most workers and still believed by a few until recently to be the only pathogenic protozoan parasite of the gut.

*Giardia* was considered to be merely one of several commensal protozoa of the gut (including *Endolimax nana, Iodamoeba butschlii, Chilomastix mesnili*, and *Trichomonas hominis)*. However, the unique morphology and size make *Giardia* relatively easy to spot under the microscope. The only feature that *Giardia* shares with *E. histolytica* is epidemiologic, i.e., they are both transmitted via a fecal-oral route, and their presence in stools indicates fecal pollution. Today *G.lamblia* has reached the ultimate as an obligate protozoan parasite of man. It is considered to be pathogenic and often but not always, elicits a wide spectrum of upper digestive tract symptoms. Its sole mode of transmission was long believed to be person-to-person via direct fecal contamination or by contaminated vegetables or other fomites.

However, we now know that *G. lamblia* or other species of the genus, are acquired from both modern municipal water supplies and from remote uninhabited mountain streams. It has recently been grouped with *Treponema, Gonococcus, Shigella,* and *E. histolytica* as a causative organism of sexually transmitted diseases (3,4).

The fact that the presence of *G. lamblia* was noted in records of surveys conducted many years ago was masked because the organism was not believed to be of sufficient importance to include in index descriptors or key words in abstracting journals.

Surveys to detect parasites of the gut were often stimulated by parasitologists or public health workers with diverse phylogenetic interests; that is, the helminthologists were concerned with the worms and the protozoologists with the protozoa. For example, reports of the numerous surveys for hookworm disease conducted under the auspices of the Rockefeller Foundation many years ago contain very little information on the prevalence of *Giardia* or other protozoa. Examinations of fresh stools for hookworm eggs and the use of sodium hydroxide treatment in the Stoll egg counting technique provide little information about the stool protozoa. Therefore, the only survey reports likely to contain accurate prevalence data for *Giardia* would be those conducted to detect *E. histolytica.*

Stool surveys have been conducted for various reasons: to assess the prevalence of organisms in recently returned servicemen from wars, ranging from World War I up to the Vietnam conflict (5-8), to recognize parasites in children in orphanages and mental institutions (9-12), in population groups following epidemics of diarrheal disease (13-15), in American Indians and Eskimos throughout the United States (10,16-21), in college students, in residents of communities with recognized poor personal or community hygiene (22-28) and in some cases for reasons not clearly stated.

To this day, a confirmatory diagnosis of giardiasis depends on the results of examining stool specimens which have been normally passed or obtained by administering cathartics. The end result is still fecal protozoology! It is, therefore, important to consider not only which populations were being surveyed and for what purpose, but also, and perhaps more importantly, what techniques were being used to assess the prevalence of the parasite.

Table 1 contains an overview of stool examination procedures, some developed in the past, and some more recent, but all utilized today. The category "L" or "other" includes a conglomeration of techniques such as simple gravity sedimentation, brine flotation, or mixtures of stool with sand to break up the fecal particles. The direct saline wet-mount technique is most widely used. For over 70 years it has been the preferred technique and is still used exclusively in many diagnostic laboratories for individual patient evaluations and for detecting infections in large populations. A skilled microscopist can use this technique to obtain much valuable information on a fresh stool specimen. Cultivation is one of the least used techniques. In fecal protozoology it is used routinely only to supplement other procedures for diagnosing amebiasis. It has not yet been widely applied in diagnosing

*Table I. Techniques - Stool Examination*
*Intestinal Protozoa*

A. Direct saline wet mount: Saline, iodine
B. Schaudinn's fixative: Hematoxylin, trichrome
C. Kohn's Chlorazol Black E
D. Merthiolate-Iodine-Formalin (MIF)
E. Phenol-Alcohol-Formalin (PAF)
F. Sodium acetate-Acetic acid-Formalin (SAF "Junod")
G. Polyvinyl-alcohol fixative (PVA)
H. Formalin (5 to 10%)
I. Zinc sulfate flotation
J. Formalin-Ether sedimentation
K. Cultivation
L. Other

giardiasis. The remaining techniques shown in Table I (B,C,D,E,F,G,H,J,) were developed to provide the laboratory with the options of permanent staining, concentration and preservation in order to overcome one of the major problems in parasite fecal diagnosis. Autolysis, disintegration, bacterial overgrowth, and all kinds of morphologic degeneration can occur if an untreated stool specimen is allowed to stand at various temperatures for a long time after it is obtained and before the diagnostician examines it.

The zinc sulfate and formalin-ether procedures evolved from efforts to improve the diagnostic capabilities of the laboratory by maximizing the recovery of protozoan cysts and helminth eggs from specimens. Unfortunately in *Giardia* detection, the formalin-ether method is questionable. There is good evidence that it concentrates *Giardia* cysts poorly, causing much distortion, and that in most cases it is not as efficient as a direct wet-mount or zinc sulfate procedure.

It is therefore important to realize that any published prevalence rates for *G. lamblia* in population surveys must be interpreted in light of the manner in which the specimen was collected, the techniques that were used, as well as the competence of the examiner.

Many years ago Svenssen and Linder(29) concluded that a series of about 10 normally passed stools must be examined per patient if at least 90% of all parasitic protozoan infections are to be detected. Later, Svenssen(30) and Sawitz and Faust(31), among others, concluded that they could reduce the number of stools examined to six and still detect from 70 to 90% of the infections. However, in order to obtain these results, at least two techniques would have to be used per stool from each patient. It is therefore quite possible that with the one-technique-per-stool approach taken in many surveys aimed primarily at detecting *E. hystolytica*, only 35 to 50% of the *Giardia* infections present were found.

All things being equal, if a freshly passed specimen is examined correctly using an appropriate procedure, and the microscopist is capable of distinguishing the various morphologic features of the protozoa, about half of the infections that exist will be detected. The advantages offered by such preservatives as merthiolate-iodine-formalin (MIF) or 10% formalin, and the advantages of a concentration method such as zinc sulfate, for example, may improve these detection rates somewhat. However, all things are not always equal and the advantages provided by a particular technique may be negated by a poor examiner or an old specimen.

Table 2 shows *G. lamblia* infections detected among children in stool surveys in five randomly selected situations. Prevalence rates of 4 to 22% were recorded. Desowitz(32) recently used wet-mount examination in detecting *Giardia* infections at a level considered to represent the normal rate (4 to 5%). However, Weiner et al(33) in Philadelphia used the polyvinyl-alchohol fixative, formalin-ether concentrate, and stained slides to document *Giardia* infection prevalence rates of 8 to 20%. Many years ago Owen et al(10) examined one stool from each of 83 Indian boys in a procedure which involved emulsifying the specimen in water and then centrifuging it and staining it with iodine. Boeck(1), on the other hand, used the simple direct wet-mount examination with iodine and found that 22% of 50 Boston children were infected. Maxey(34) found that 16% of 89 children were infected. The procedure he used was probably a direct wet-mount, but he does not outline the exact technique in his paper.

*Table 2.* Giardia lamblia - *Children - Stool Surveys*

| Place | Ethnic group | No. Specimens | % Positive | Reference |
|---|---|---|---|---|
| Hawaii | Local | 275 | 4 | (32) |
| | Foreign | 115 | 5 | |
| Philadelphia | Puerto Rican | 167 | 20 | (33) |
| | White | 169 | 8 | |
| | Black | 49 | 8 | |
| Wyoming | Indian | 83 | 22 | (10) |
| Boston | Local | 50 | 22 | ( 1) |
| Baltimore | Local | 89 | 16 | (34) |

The survey by Boeck(1) is noteworthy for at least two reasons: First, it was designed primarily to detect *Giardia* infections; second, he found no other protozoa in the stools from these 50 children. Boeck concluded that the available evidence did not support the hypothesis that *G. lamblia* itself caused diarrhea in either children or in adults, except perhaps in a few obscure and sporadic cases.

The variability of *G. lamblia* infection rates as detected in several surveys conducted primarily on adults is shown in Table 3. Various techniques were used to document prevalence rates ranging from 2 to 15%. Boeck and

Stiles(35) evaluated more than 8,000 specimens by direct examination only. Brooke and his coworkers(15) used polyvinyl-alcohol fixative, stained slides, formalin preservation, and, in some cases, formalin-ether concentration. Ballinger(36), using direct examination, zinc sulfate flotation, and, with some of the specimens, water sedimentation, detected a low 2% infection rate among hospital employees.

*Table 3.* Giardia lamblia - *Stool Survey - Adults.*

| Place | No. Specimens | % Positive | Reference |
|---|---|---|---|
| Washington, D.C. | 8,029 | 7 | (35) |
| South Bend, Ind. | 600 | 4 | (15) |
| New York, N.Y. | 427 | 2 | (36) |
| Aspen, Colo. | 419 | 5 | ( 2) |
| Atlanta, Ga. | 4,000 | 5 | (38) |
| Minneapolis, Minn. | 169 | 15 | ( 8) |

The stool survey conducted in Aspen by Gleason et al(2) was another in which a 5% *Giardia* infection rate was detected among a resident population following an epidemic in that city(37). Although such an infection rate seems high for this population group, it probably results from the fact that Gleason and colleagues used chlorozol black, polyvinyl-alcohol fixative, trichrome stain, plus formalin preservation and formalin-ether concentration. They used virtually every currently known technique except for MIF and zinc sulfate for detecting *Giardia*. After World War II, Jacobs and his coworkers(38) found a 5% *Giardia* infection rate in a stool survey on 4,000 military personnel. They did direct examination, zinc sulfate flotations, and in some cases, evaluated stained slides. In the temperate climate of Minneapolis, Riley(8) used direct wet-mount examination, and in some cases, stained slides in detecting a 15% *Giardia* prevalence rate. As can be seen from the results of these modern surveys, prevalence rates for *G. lamblia* vary with population group, time, and the investigator.

Detecting the presence of *Giardia* in stools is made more difficult by its variable excretion pattern. When conditions are ideal for the "biologic production" of this organism, it seems to outdo itself in fecundity. Many years ago Tsuchiya(39) found *Giardia* in virtually all of the stool specimens collected from two infected individuals over several days. The highest counts he recorded were 7.05 x $10^8$/day for one subject and 5.85 x $10^8$/day for the other. Porter(40) had previously documented cyst excretion counts in patients as high as 1.44 x $10^{10}$/day. Kofoid and Swezy(41) reported cyst counts of as high as 3.9 x $10^9$/day excreted by one individual. Such *Giardia* concentrations should clearly provide enough organisms to be detected with any accepted procedure applied to the 2 to 3 stools produced per day by the average person. However, as stated earlier, excretion rates vary substantially.

Rendtorff(42) showed one of the best-illustrated examples of the variable excretion patterns of *Giardia* and other protozoa in his carefully conducted human volunteer study reported in 1954. Danciger and Lopez(43) examined stool specimens from hospitalized children and provided one of the most interesting examples of the variability of *Giardia* excretion.

From their studies, children could be divided into three groups on the basis of their level of *Giardia* excretion: those who were high excreters; low excreters; and mixed excreters. Those with high rates of excretion expelled up to 7,000 *G. lamblia*/mg feces and regularly excreted as many as 1,000 cysts/mg feces for several days. Those with low excretion rates varied greatly in the excretion of cysts, having as few as 12 to 108 cysts/mg feces with periods of several days during which no organisms were detected. On the other hand, those with the mixed excretion rates had periods when no organisms could be detected in stools and other periods when 400 to 900 cysts/mg feces were present.

The results of multiple stool examinations for *Giardia* performed by other workers are shown in Table 4. Boeck and Stiles(35) found an 8% infection rate during their initial examination and found a 15% infection rate after 6 examinations. Jeffery(44) in his excellent report on the 3-year study of parasitism in a mental institution described a 5% infection rate detected by a single examination, and a 35% rate detected after 9 examinations. Indeed, in a 7-year-study on 61 patients, he documented a 33% prevalence rate after examining 13 specimens(45). Borland(46) found a 3% prevalence rate on his first examination of stools from an adult population but found a 10% prevalence rate after the third examination.

*Table 4. Repeat Stool Examinations* - Giardia Lamblia

| Number Specimens | % Positive on Initial Exam | % Positive on Multiple Exams (No. Exams) | Reference |
|---|---|---|---|
| 505 | 8 | 15 ( 6) | (35) |
| 110 | 5 | 35 ( 9) | (44) |
| 61 | 10 | 33 (13) | (45) |
| 191 | 3 | 10 ( 3) | (46) |

Results of a comparable study done in our laboratory are shown in Table 5, which illustrates the bimonthly prevalence of intestinal protozoa in 78 children whose stool specimens were examined six times in a year. This study was done following the initial survey(21). We used direct wet-mount examination and formalin-ether concentration on all specimens. PVA-fixed, trichrome-stained slides were examined when the stools were soft, loose, or watery. Individual bimonthly prevalence rates of 5 to 16% were obtained. However, for the whole study year 19 (24%) of these children were infected at some time.

*Table 5. Prevalence of intestinal parasites in 78 Cherokee Indian second grade school children*

| Parasites | Prevalence at Each Month (%) May'66 | July'66 | Oct '66 | Dec. '66 | Feb. '67 | Apr. '67 | Total Prevalence for 6 Months (%) |
|---|---|---|---|---|---|---|---|
| E. histolytica | 5 | 9 | 9 | 12 | 11 | 1 | 14 |
| E. hartmanni | 22 | 13 | 10 | 15 | 23 | 13 | 38 |
| E. coli | 36 | 45 | 33 | 36 | 35 | 35 | 58 |
| E. nana | 24 | 18 | 18 | 18 | 23 | 23 | 41 |
| G. lamblia | 16 | 15 | 5 | 8 | 11 | 7 | 24 |
| C. mesnili | 8 | 5 | 4 | 5 | 4 | 1 | 10 |

The results obtained on stool specimens positive for *Giardia* from each of the 19 children can be seen in Table 6, which also contains the findings from a May 1965 survey. In some instances, we detected *Giardia* in every stool specimen obtained from a particular child (No. 37 and 62). In some instances we found organisms in only about half of the stools collected from a child (No. 39), with some *Giardia* found in specimens obtained early in the study and some found near the close of the study. In a few instances, only 1 specimen out of the 7 obtained from a particular child was positive (No. 61 and 95).

What do these findings mean? In addition to the difficulty of consistently detecting *G. lamblia* organisms in stool surveys conducted in the United States in the last 60 years, there is the problem of assessing the relevance of these findings to the true prevalence of disease in the population. Survey specimens merely show the surface of the real presence of the parasites in individuals. Economic, time, and personnel constraints realistically prevent our performing the examination of at least 6 specimens per individual deemed adequate to detect infections. It is not even feasible to examine the

*Table 6.* Giardia lamblia *detection in stool specimens of 19 Indian school children*

| Examination Date | Child No. 14 | 15 | 24 | 29 | 37 | 39 | 44 | 48 | 50 | 53 | 55 | 56 | 58 | 61 | 62 | 65 | 82 | 95 | 201 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| May, 1965 | X | X |  | X | X | X | X | X |  | X |  | X | X | X | X |  | X |  | X |
| May, 1966 | X | X |  | X | X | X |  |  | X | X |  | X |  |  | X |  | X | X | X |
| July, 1966 | X |  |  | X | X |  | X |  |  | X |  |  |  |  | X |  |  |  |  |
| Oct. 1966 |  |  |  |  | X |  |  |  |  | X |  |  |  |  | X | X |  |  |  |
| Dec. 1966 | X |  |  | X | X |  | X |  |  |  | X |  |  |  | X |  |  |  |  |
| Feb. 1967 | X |  | X | X | X | X | X |  |  |  |  |  | X |  | X |  |  |  |  |
| April, 1967 | X |  |  | X | X | X | X |  |  |  |  |  |  |  | X |  |  |  |  |

X = Organisms Detected

recommended 3 stools (the standard O and P x 3) in surveys. Indeed, evidence in the clinical literature on individual patients with symptoms suggestive of giardiasis, particularly that contained in the study by Kamath and Murugasu(47) and the review by Burke(48), indicates that a number of stool examinations may still be negative when it is possible to find *Giardia* aspirated in duodenal contents as well as in biopsy material or mucosal imprint smears.

In the future, I think we must develop more sophisticated techniques to detect *Giardia* in the population. Our present technology, for example, of using the filter developed by the Environmental Protection Agency (see Jakubowski, these Proceedings), shows that we can probably more accurately detect a few cysts in 20,000 gallons of water than we can in 2 g of feces from an infected individual. I believe that the future of stool examination in general depends upon the development of more sensitive techniques which do not depend solely on the morphologic identity of an organism. The recent report by Root and his coworkers in Mexico(49) on using an enzyme-linked immunosorbent assay (ELISA) procedure for detecting *E. hystolytica* antigen in stool specimens is exciting. If we can adapt this technique and rely on its results in detecting *G. lamblia* in stool specimens rather than on the morphology of the variably excreted organism, we will have reached a milestone in enlarging our diagnostic capability.

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## Discussion

C. HENDRICKS: There are some very good data to show that giardiasis can be a waterborne disease, but I think it is obvious that giardiasis can be transmitted by person-to-person contact as well as foodborne. In your opinion, to what extent do you think giardiasis may be transmitted by water, and can you perhaps give some idea on what the degree of national concern should be?

G. HEALY: I am a diagnostician and you may want to hear from some of the epidemiologists who have a more intimate knowledge of the epidemiology of *Giardia*. The evidence is certainly strong that it is transmitted by water: it has been found in water and it has been associated with diarrheal diseases in surveys that have been conducted that indicate that it was transmitted through water. I think there should be national concern for it.

M. SCHULTZ: The evidence for waterborne transmission will be presented in papers by Dr. Juranek and Mr. Craun; if it is not, I will ask the question again. But, I would like to challenge you on your comment that there is evidence that giardiasis is foodborne. I do not know of any such evidence.

G. JACKSON: As far as I know, there are no proven cases of foodborne giardiasis. The cysts have been detected, presumably in a viable state, on strawberries, but we have no direct evidence of a human case. I only saw a newspaper story on this; however, in New York City, venereal transmission has been implicated in giardiasis.

M. WOLFE: I do not know of any proven evidence that *Giardia* is transmitted by food. It just stands to reason that people travelling in endemic areas who are eating salads or breads, or are drinking beverages that have been handled by infected food handlers, may become infected this way. I do not think that giardiasis can be considered exclusively a waterborne disease. The epidemiologic evidence in this country and from the Soviet Union certainly indicates that this is the major form of transmission. I really cannot see it as the exclusive way that *Giardia* is transmitted. I believe it is reasonable to say that a salad, mixed by fingers of an infected food handler, after it has been washed in water, constitutes a foodborne rather than a waterborne disease.

R. RENDTORFF: It is very important to consider this parasite in terms of past and present. I think the survey that you gave, Dr. Healy, is very important in this regard. I too feel that *Giardia* can be transmitted by a number of routes, as are all parasitic protozoa. We tend to focus here on *Giardia* and forget that there are other organisms of very similar size that are also transmitted and have the same biological characteristics as *Giardia*. In the future you may pay more attention to these, such as *E. coli* and *Entamoeba histolytica*, in terms of how they might be transmitted through water.

One thing that bothers me about the problem with giardiasis today is the fraction of physicians who obtain a report from hospital laboratories of *Giardia lamblia* in a patient when there is no symptom referable to giardiasis. Should this patient be treated or shouldn't he be? It is an expensive medical, economical problem. Giardiasis is fairly prevalent in our population, as you can see by these statistics. If five per cent of us have it, it takes a great deal of Atabrine (quinacrine) and a lot of treatment to get rid of this organism from society, and we do not know whether we are really getting rid of it this way.

I believe we have to interpret these statistics very carefully. Years ago, when we did our experiments, the medical profession at large thought *Giardia* was perfectly non-pathogenic, and it was rarely treated. In fact some hospitals did not report what they considered to be non-pathogenic species. If you look at hospital statistics, I think you will find that in years past, *Giardia* was considered to be non-pathogenic and non-existent, while *E. histolytica* was everywhere. One was overreported and the other was underreported. Accordingly we must carefully interpret these data.

R. DAVIES: There is a published report of foodborne giardiasis by Gangarosa (Journ. Inf. Dis., 122:354, 1970.) His only data were derived from one outbreak, and I believe it affected about 19 people. There was no explanation in his text. That is the only published report I have ever seen in reference to foodborne giardiasis. (*Editor's Note:* The article referred to by Mr. Davies was adapted from the then National Communicable Disease Center (NCDC) Foodborne Outbreak Annual Summary for 1969. At that time, waterborne outbreaks were incorporated into the foodborne summary. Although not mentioned in the Journ. Inf. Dis. paper, the NCDC summary has water listed as the vehicle in this outbreak.)

D. JURANEK: We have had one foodborne outbreak reported in the CDC Annual Summary of Foodborne Outbreaks, 1973; but, as I recall, the epidemic was worked up inadequately. Although the epidemiologist concluded that the epidemic may have been the result of foodborne transmission, not much manpower or effort was devoted to the investigation and the data are far from conclusive. Had the epidemiologist realized the importance and controversial nature of his observations, I am sure that the epidemic would have been worked up better.

S. ERLANDSEN: You are talking about a prevalence of about 24 per cent on cases in your stool surveys. If we look at some of the animal literature two studies come to mind: one performed in Czechoslovakia and one performed in Japan, on essentially opposite sides of the world. They refer to prevalence of *Giardia* in animals on the order of 70 to 85%. Would you think perhaps that we can see a higher percentage than your 24% in terms of trophozoites being normally found in the bowel but not showing any signs of symptomatology, just a normal inhabitant?

G. HEALY: I think the prevalence rates would probably be much higher if we had better techniques for detecting the organisms. We have to identify them in stools, as trophozoites, or we have to look for the median bodies in the quadrinucleate cyst form, and we have been doing the same thing now for 75 years. Our technology has reached the point where we probably should utilize some of the more sophisticated techniques. The ELISA technique for antigen in stools is one that I think is especially good. Mr. David Root (Millipore Corp., Bedford, Mass.) came with us when we did a survey of Cherokee Indians this past year and showed us how this technique worked for *E. histolytica* infection. With this technique, we detected *E. histolytica* antigen in 2 Indian children. Our regular stool examination techniques had shown that one was negative on initial examination.

The ELISA test may be one way to establish the prevalence of the organism by obtaining an index of its presence by antigens or proteins, because they get broken up on the way down through the bowel. Our detection system is quite poor in detecting organisms in very small numbers, whereas presumably a small amount of protein could be picked up by the ELISA test.

T. VERNON: A comment on the generalization of prevalence surveys: it concerns me that we would leave here with the figure of five per cent. I think it important to note that in the prevalence studies that have been done we have not adequately characterized the survey population. Certainly the Aspen residents with their 5% prevalence in the spring of 1966 were not typical of the residents of the United States in general. The Cherokee Indians in North Carolina are certainly not representative, either. Since that 5% figure has been used rather frequently, I wanted to comment on that.

Incidentally, I did catch the tense of the verb you used when you said the Aspen residents "are" drinking water contaminated with *Giardia* cysts. Whatever is happening in other ski resorts, I do not know, but it certainly should be stated, and the Aspen residents would agree, that Aspen has well-filtered water.

G. HEALY: The situation was corrected. In reference to the prevalence rate mentioned in those instances, I do not know what the prevalence of *Giardia* is throughout the United States, and I could not give you a figure. It would depend on which population you wanted to pick and how representative that population is of the entire country. It does vary from place to place.

# Animal Reservoirs and Cross-Species Transmission of *Giardia**

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## ABSTRACT

During 1975-77 a survey of wild and free-ranging domestic animals and man for *Giardia* was completed in several areas of Colorado. Fecal samples obtained from animals and man were examined by a zinc sulfate centrifugation technique. Of 744 samples from 33 species of vertebrates, 65 (9%) were positive for *Giardia*. Six species of mammals were represented with positive findings as follows: beaver (*Castor canadensis*) 44 of 244 (18%); coyote *(Canis latrans)* 2 of 34 (6%); cattle *(Bos tarus)* 6 of 58 (10%); domestic cat *(Felis domesticus)* 1 of 4 (25%); dog *(Canis familiaris*) 10 of 78 (13%) and man *(Homo sapiens)* 2 of 32 (6%). Human source *Giardia* cysts were given to laboratory animals, wild animals and domestic animals. Hamsters *(Mesocricetus auratus)*, domestic rabbit *(Oryctolagus cunniculus)*, laboratory mice *(Mus musculus)*, deer mice *(Peromyscus maniculatus)*, black bear *(Ursus americanus)*, wapiti *(Cervus canadensis)*, mule deer *(Odocoileus hemionus)*, white-tailed deer *(Odocoileus virginianus)*, domestic sheep *(Ovis aries)*, and cattle *(Bos tarus)* did not become infected. Laboratory rats *(Rattus norvegicus)*, gerbils *(Gerbillus gerbillus)*, guinea pig *(Cavis porcellus)*, beaver, dog, raccoon *(Procyon lotor)*, bighorn X mouflon sheep *(Ovis canadensis X O. musimon)*, and pronghorn *(Antilocapra americana)* became infected with *Giardia*. Cysts were first found in the feces from 6 to 34 days post exposure. Animals were positive for *Giardia* for from 1 day to 3 months.

The flagellated protozoan *Giardia* (Protozoa: Hexamitidae) was first observed by Leeuwenhoek, who found the trophozoite in his own feces, and was later described by Lambl in 1859(1,2). Europeans often call the parasite *Lamblia intestinalis*, but *Giardia lamblia* is the preferred and generally accepted name for the human form(3). A recent survey(4) has shown that *G. lamblia* is the most common parasite of man in the United States, infecting about 3.8% of the populace with prevalence ranging from 2 to 20 percent(5). *Giardia* is about 3 times more common in children(6) and up to 100% of a group of children may be infected(1). The pathogenicity of *Giardia* is a controversial subject(6) in spite of numerous reports of *Giardia* caused diarrhea(2,7,9) and one report of death(10).

*Giardia* infections are acquired by ingesting viable cysts(1). Cysts may be ingested on or in food(1,11), in water or during intimate contact(1). Several outbreaks of giardiasis have been traced to municipal water supplies(5,9,12, 16). Two of these, in Aspen(12) and Vail(14), Colorado, were traced to contamination of the water supply by sewage. An outbreak of giardiasis in Camas, Washington has been associated with *Giardia* infected beaver living in streams supplying the Camas water system(17). Raw water samples from the water system of Rome, New York, collected during an outbreak of giardiasis, were fed to beagle puppies; infection with *Giardia* resulted in experimental dogs while control dogs remained negative(15). These cysts would probably infect humans as well. Previous work completed at Colorado State University (CSU) has shown human-source *Giardia* will infect dogs and cats and circumstantial evidence indicates the reverse also occurs (Hibler, unpublished data).

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Giardiasis exists in 2 forms in Colorado: endemic and epidemic. During the last 5 years an average of 280 cases of human *Giardia* have been reported each year; there have been 271 reported cases in 1978 as of 26 August and 75 of these occurred in the month of August(18). Epidemics of giardiasis have been reported from Aspen(12), Boulder(19), Vail(14), Estes Park(5) and various resort lodges(20,21). In most cases the source of the cysts was not determined but the possibility of animal reservoirs being the source has been raised(20).

Very little cross transmission work has been done with *Giardia*. Previous work has consisted of infecting laboratory rats and woodrats *(Neotoma fuscipes)* with *Giardia* obtained from man(6). Grant and Woo(22) found that *G. muris* from laboratory mice, *G. simoni* from laboratory rats and *G. peromysci* from deer mice were very host specific while *G. microti* and *G. mesocricetus* were not host specific. Human-source *Giardia* has been given to puppies and infections with *Giardia* have been established(23).

Due to the lack of cross transmission work and the belief that *Giardia* is very host specific, different specific names have been given to *Giardia* from each host(6). Filice(24) compared some species of *Giardia*, found no structural difference and concluded there were two species of *Giardia* which infect mammals: *G. muris* in the mouse, rat and hamster and *G. duodenalis* in all other mammals including man.

Because of the high number of cases and epidemics of human giardiasis in Colorado and the possibility that wild or domestic animals infected with *Giardia* contributed to these outbreaks, the present study was begun in 1975. The study consisted of two parts: 1) a survey of wild and free-ranging mammals, including man, along streams in areas which were both endemic and had a history of epidemics of giardiasis, and 2) an attempt at cross-transmission utilizing human-source *Giardia* to infect as many species of laboratory animals, wild animals and domestic animals as possible.

## METHODS AND MATERIALS

### Survey of Wildlife

Fecal specimens were obtained from water, off the ground, from the rectum of animals shot or killed by automobiles, from veterinary clinics, and from backpackers and their pets. The greatest number of samples was obtained by picking up fresh feces of various animals. Samples were not picked up unless they were less than 12 hours old except for those of beaver which were collected from the bottom of ponds. Beaver feces remained cool and moist in these ponds. Dry or frozen feces were not collected. Samples also were obtained from animals submitted for necropsy at the Wild Animal Disease Center, CSU. Additional fecal samples were obtained from animals live trapped and released or kill trapped. Fecal samples from dogs and cats were obtained from veterinarians in Aspen and Granby, Colorado and from the ground in the wild.

A survey of backpackers and their pets utilizing the Maroon Bells-Snowmass Wilderness Area near Aspen, Colorado (Fig. 1) was completed in 1976. Each backpacker going through the U.S. Forest Service check station on the Maroon Bells road was given a packet which included an explanation of the study, a 60 ml polypropylene bottle containing 40 ml of 2% formalin solution and a prepaid and addressed envelope. The backpacker was asked to place approximately a teaspoon of feces into the bottle, mark the label as to sex, age, and species of the source, and mail the bottle to the Wild Animal Disease Center, CSU. Results of the fecal examinations were made available to interested cooperators. Fecal samples collected in 1975 were placed in a solution of 10% formalin. This method did not prove entirely satisfactory as 10% formalin disrupted many of the cysts. In 1976, samples were placed in 2% formalin or refrigerated in distilled water until examined.

Samples preserved in formalin were shaken to suspend all the particles and a 15 ml round bottom centrifuge tube was filled. After centrifuging at 2300 rpm for 5 to 10 min, the supernatant was decanted and the sediment examined. Samples in distilled water were treated the same as formalin-fixed samples if excess water was present; if not, a marble sized piece of feces was used for examination. All fecal samples were examined by a zinc sulfate ($ZnSO_4$) centrifugation technique.

$ZnSO_4$ at a specific gravity of 1.18 is made by adding 331 g of $ZnSO_4$ to 1000 ml of water; since $ZnSO_4$ is hygroscopic, 331 g is seldom sufficient and a hydrometer must be used to adjust the specific gravity. Lugol's iodine is made by adding 10 g of potassium iodide and 5 g of iodine to 100 ml of distilled water.

Five or 6 drops of Lugol's iodine is added to the sediment in the centrifuge tube and thoroughly mixed. The tube is then filled half way with $ZnSO_4$ solution and thoroughly mixed. $ZnSO_4$ solution is added until the meniscus bulges slightly and a coverslip is placed on top. Centrifuge at 2300 rpm for 3 min, remove the coverslip and place on a slide for microscopic examination.

## THE SURVEY STUDY AREAS

Aspen and Hideaway Park in central Colorado (Fig. 1) were chosen as the primary study areas because of a history of endemic and epidemic human giardiasis in each area, and their year-round use by a large number of people.

Aspen is a small resort town in Pitkin County at an elevation of 2400 m (7900 feet). Tourism during the summer and skiing during the winter months are the main industries. The surrounding terrain is very steep and mostly covered with trees or brush. Aspen utilizes water from Castle and Maroon Creeks for the city water supply.

Hideaway Park is a small resort town in Grand County at 2900 m (8800 feet) in elevation. It is the nearest town to the Winter Park Ski Area, which has no overnight or restaurant facilities. Therefore, Hideaway Park is a center of activity in the winter. During the summer it is a popular resort. The water supply for Hideaway Park is the Little Vasquez River. The city of Denver, Colorado, also takes water from the Vasquez River Basin. Several secondary sampling areas near Hideaway Park also were examined. These river drainages included Beaver Creek, Cow Creek (Cow Gulch), Williams Fork River, Willow Creek, Corral Creek and the Fraser River.

Brush Creek, near Eagle in west-central Colorado (Fig. 1), was sampled after a human who drank water from East Brush Creek developed giardiasis 9 days later. Both the east and west branches of Brush Creek flow through private and public property. Fishing and camping are the main activities in the area.

Vermejo Park is a large (193,500 ha or 480,000 acre) ranch in north-central New Mexico owned by the Pennzoil Corporation. There is little human activity on the ranch. Fecal samples were gathered from several species of animals which would have little chance of acquiring the parasite from man. A small part of Rocky Mountain National Park (RMNP) was examined because an outbreak of giardiasis in 8 people was reported(5). The people were from California and stayed at the Cascade Cabins on Fall River within the boundaries of RMNP. Fecal samples, from animals, found along Fall River to its origin and from along Hidden Valley Creek, were examined.

Samples from animals from the entire state of Colorado were examined whenever they became available. These are all listed under the heading "Statewide".

## EXPERIMENTAL INFECTIONS

Human feces containing *Giardia* cysts were obtained from the CSU Student Health Center, Poudre Valley Memorial Hospital and the Northern Colorado Diagnostic Laboratory. Beaver-source *Giardia* were obtained from naturally infected beaver on Beaver Creek near Hot Sulphur Springs, Colorado. Mule deer source *Giardia* were obtained from a naturally infected fawn (part of a captive herd), near Kremmling, Colorado.

A small amount of feces from each sample was examined by the $ZnSO_4$ centrifugation method to ensure the presence of *Giardia* cysts. If the cysts appeared viable, the entire fecal sample was thoroughly mixed with distilled water. This mixture was strained through a double thickness of cheese cloth, the container rinsed with distilled water, and the fluid poured over the filtered feces which was then discarded. The filtered liquid was poured into 50 ml round bottom test tubes and centrifuged at 2300 rpm for 5 minutes. The supernatant was discarded and the pellet resuspended in distilled water and centrifuged again. This process was repeated until the supernatant was almost clear. The fluid was discarded and once again the pellet resuspended in distilled water. This was allowed to stand for 1-5 minutes or centrifuged at 1000 rpm for 10 seconds to sediment larger particles. The supernatant was then poured into a glass beaker and samples taken to determine the

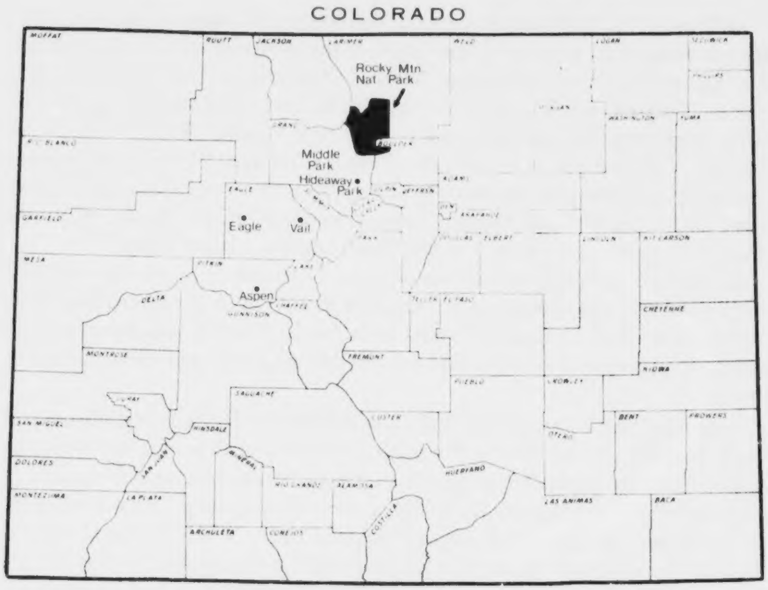

FIG. 1. *Locations of* Giardia *survey study areas in Colorado 1975-1977.*

concentration of *Giardia* cysts. Fluid could be removed, after the cysts settled to the bottom of the beaker, or added to obtain the desired number of cysts per volume of fluid.

Animals were infected via stomach tube, orally or in water provided to animals deprived of water for the previous 24 hours. Ten thousand cysts were used as an infective dose whenever possible. Fecal samples were examined daily from each animal, including controls, for a minimum of 14 days prior to infection and every day after infection for at least 21 days. All fecal samples were examined by the $ZnSO_4$ centrifugation method. Animals were monitored to determine the prepatent period (the time from infection to the passing of cysts in the feces) and the patent period (the length of time cysts were found in the feces).

Laboratory mice and rats, hamsters, guinea pigs, gerbils and domestic rabbits were obtained from the Experimental Animal Service at CSU or local pet shops. Beaver, raccoon, deer mice and house mice were live trapped in the wild. The large wild mammals, mule deer, white-tailed deer, wapiti, pronghorn, bighorn X mouflon sheep and black bear were obtained from the Colorado Division of Wildlife or were captive animals held in pens at the CSU Foothills Campus. Large domestic animals (sheep and cattle) were obtained and held in pens at the Foothills Campus. Specific Pathogen Free (SPF) beagle puppies were obtained from the Collaborative Radiological Health Laboratory (CRHL) on the Foothills Campus. All animals were kept either at the Foothills Campus or in animal rooms on the main campus, fed dry commercial feed and/or alfalfa hay. Fort Collins city water was provided as needed.

When feasible, animals were sacrificed and wet impression smears and smears from intestinal scrapings were made from various levels of the intestine. Wet smears were stained with Lugol's Iodine and examined immediately. A fecal sample was taken from the lower colon at necropsy and examined by the $ZnSO_4$ centrifugation method.

## RESULTS OF THE SURVEY

### Overview

One of the primary objectives of the study was to determine if wild and/or free ranging mammals found along streams utilized by municipalities and outdoor recreation users for drinking water, were infected with *Giardia*. As the primary complaints of giardiasis were from backpackers who thought they acquired the parasite while backpacking, most samples collected during 1975 were near or above timberline. Samples were taken at lower elevations during 1976.

A total of 744 fecal samples from 33 species of vertebrates was examined by the $ZnSO_4$ centrifugation method (Tables 1 and 2). Sixty-five of the 744 fecal samples (9%) were positive for *Giardia* with positive findings in mammals as follows: beaver 18% (44 of 244); coyote 6% (2 of 34); cattle 10% (6 of 58); domestic cat 25% (1 of 4); dog 13% (10 of 78) and man 6% (2 of 32).

During 1975, 164 samples were examined from three areas: Aspen, Middle Park and Vermejo Park. Cattle (5 of 23), cats (1 of 2) and dogs (7 of 19) were infected with *Giardia* (Table 1). Most of the 1975 samples were recovered at high altitudes (over 2700 m or 9,000 feet). In 1976, sampling was concentrated at lower elevations and 52 of 580 fecal samples were positive for *Giardia* (Table 2). These were beaver (44 of 244), coyote (2 of 34), dogs (3 of 59), cattle (1 of 35) and human (2 of 31).

### Aspen

Aspen utilizes Castle and Maroon Creeks for its city water supply. Samples from these streams and their major tributaries above the city water siphons, and fecal samples from animals in the vicinity of the streams were examined.

Maroon Creek is a typical mountain stream with fast flowing water. The terrain on both sides is steep and covered with various types of vegetation. Willow (*Salix* spp), aspen (*Populus* spp), pines (*Pinus* spp), spruce (*Picea* spp), firs (*Pseudotsuga*) and juniper (*Juniperus* spp) are the main species seen with numerous grasses and forbs present as an understory. Many slopes are in reality cliffs and numerous talus slopes are present. Some open grass covered avalanche paths are seen.

Aspen takes water from Maroon Creek, 270-360 m (300-400 yards) above the T Lazy 7 Ranch. As far as is known, there are no human dwellings above the T Lazy 7 Ranch along Maroon Creek. Numerous campgrounds are maintained by the U.S. Forest Service along Maroon Creek up to and including Maroon Lake. These campgrounds are used heavily all summer.

*Table 1. Number of fecal samples of New Mexico and Colorado mammals examined for* Giardia *sp. in 1975-1976.**

| | AREA | | | |
|---|---|---|---|---|
| Animal** | Vermejo Park | Aspen | Middle Park | Total |
| Colorado chipmunk | | 4 | 6 | 10 |
| Cottontail rabbit | | 1 | | 1 |
| Golden-mantled ground squirrel | | 1 | | 1 |
| White-tailed jackrabbit | | | 1 | 1 |
| Marmot | | 5 | 5 | 10 |
| Pika | | 10 | 7 | 17 |
| Porcupine | | 1 | 1 | 2 |
| Richardson's ground squirrel | | 2 | 5 | 7 |
| Red squirrel | | | 1 | 1 |
| Rock squirrel | 2 | | | 2 |
| Badger | | | 2 | 2 |
| Black bear | | | 1 | 1 |
| Pine marten | | 1 | | 1 |
| Raccoon | | | 3 | 3 |
| Red fox | | | 1 | 1 |
| Striped skunk | | 1 | | 1 |
| Wapiti | 10 | 2 | 6 | 18 |
| Mule deer | | 1 | 1 | 2 |
| Bovine | | 10 | 13(5)*** | 23(5) |
| Cat | | 1 | 1(1) | 2(1) |
| Dog | | 19(7) | | 19(7) |
| Horse | | 10 | 8 | 18 |
| Sheep | | 20 | | 20 |
| Human | | 1 | | 1 |
| | 12 | 90(7) | 62(6) | 164(13) |

* *1 July 1975 - 30 June 1976.*

** *See Appendix A for a list of Scientific Names.*

*** *Number fecal samples examined (number infected).*

*Table 2 - Number of fecal samples of New Mexico and Colorado mammals examined for* Giardia *sp. in 1976-1977.**

| | AREA | | | | | |
|---|---|---|---|---|---|---|
| Animal** | Aspen | Middle Park | RMNP | Brush Creek | Other | Total |
| Beaver | 64(8)*** | 107(33) | 41 | 32(3) | | 244(44) |
| Cottontail rabbit | | | | | 1 | 1 |
| Marmot | 1 | | | | | 1 |
| Muskrat | 4 | 1 | 4 | | 12 | 21 |
| White-tailed prairie dog | | | | | 1 | 1 |
| Richardson's ground squirrel | 2 | | | | | 2 |
| Black bear | | | | | 1 | 1 |
| Bobcat | | | | | 1 | 1 |
| Coyote | | | | | 34(2) | 34(2) |
| Mountain lion | | | | | 6 | 6 |
| Raccoon | | 13 | 2 | | 9 | 24 |
| Striped skunk | | 2 | | | | 2 |
| Bighorn sheep | | | 20 | | 32 | 52 |
| Wapiti | 3 | | 6 | | 6 | 15 |
| Mule deer | | | | | 35 | 35 |
| White-tailed deer | | | | | 1 | 1 |
| Bovine | 2 | 22 | | 11(1) | | 35(1) |
| Cat | | 2 | | | | 2 |
| Dog | 20(3) | 38 | 1 | | | 59(3) |
| European ferret | | | | | 1 | 1 |
| Horse | | | 1 | | | 1 |
| Human | 30(2) | | 1 | | | 31(2) |
| Brook trout | | | 10 | | | 10 |
| | 126(13) | 185(33) | 86 | 43(4) | 140(2) | 580(52) |

* *July 1976 - 30 June 1977.*

** *See Appendix A for a list of Scientific Names.*

*** *Number of fecal samples examined (number infected).*

The area around Maroon Lake and Crater Lake are heavily used by day hikers and backpackers.

Very little wildlife was observed along the streams serving as the water supply for Aspen. Beaver were not seen above the confluence of East and West Maroon Creeks. One old beaver colony exists at Maroon Lake but no beaver were observed; however, several dams were found below the junction of the creeks. One of 29 (3.4%) beaver samples from Maroon Creek was positive for *Giardia*. None of the 4 muskrat samples from the pond where the positive beaver sample was found contained *Giardia* cysts. Most species of wildlife were found in rocky areas which were not close to open water or moist areas. Chipmunks, pika and marmot, were usually found high on the slopes in rocks or on dry ground. Marmot were found close to Maroon Creek only one time. All samples collected from these animals were negative. Few mule deer or wapiti were seen along the river and all samples from these animals were negative. On one occasion approximately 200 domestic sheep were grazing at West Maroon Pass. Twenty samples from these animals were negative for *Giardia*. None of a small herd of cattle, pastured near the confluence of East and West Maroon Creek, was positive for *Giardia*. The most common animal seen was the domestic dog. Dog feces was common around campgrounds, Maroon Lake and most hiking trails which follow rivers and creeks in the area.

Castle Creek is very similar to Maroon Creek except the valley bottom is wider. Human dwellings are found along Castle Creek and most of its tributaries. At the end of the road on Castle Creek is a mining operation and trespass beyond this point is not allowed. Approximately 0.5 km (0.3 miles) below the mine Pine Creek joins Castle Creek. A moderately used trail leads along Pine Creek to Cathedral Lake. Animal fecal samples were collected along Pine Creek and around Cathedral Lake. All pika, marmot, pine marten and dog samples from the Pine Creek area were negative. Samples from one beaver pond on Pine Creek were negative as were samples from a beaver pond just below the confluence of Pine and Castle Creeks. All animal samples collected above the confluence of Pine Creek and Castle Creek were negative. A small campground with picnic tables is located near Cathedral Lake and an outdoor toilet has been built about 60m (200 feet) uphill from Pine Creek. Below the confluence of Pine Creek and Castle Creek is an assortment of restaurants, dude ranches and private homes.

Owners of the Ashcroft Ski Touring Company would not allow collection of samples from the beaver ponds or other animals on the property. This organization had a dog kennel which was built along Devaney Creek. There is also an outhouse built along the creek near the old Ashcroft townsite. Below the Ashcroft property 22% (7 of 32) of beaver samples were positive for *Giardia* whereas none of 3 samples collected upstream from the property were positive. Express Creek flows into Castle Creek just below the Ashcroft property and originates near the summit of Taylor Pass. This is a popular "four wheel drive" road. Several houses have been built along Express Creek and several campgrounds, without sanitary facilities, are found along the

Creek above the homes. All fecal samples from pika and marmot in this area were negative for *Giardia*. One human stool found along Express Creek was negative for *Giardia*.

Conundrum Creek joins Castle Creek 9.5 km (6 miles) below Express Creek. Conundrum Creek, in part, originates from the Conundrum Hot Springs which is a very popular site in the area. It has become so popular the Forest Service has prohibited camping within 1.6 km (1 mile) of the springs. The terrain along Conundrum Creek is very steep and clifflike along most of the trail to the hot springs. There are also some very heavily timbered areas, mostly spruce and fir, and some willow patches. Very little wildlife, consisting of a small number of pika and marmots was observed along the trail. Dog feces was found along the trail and human feces, too old for sampling, was found near several old campsites often within 0.6 m (2 feet) of the streams. The area surrounding the hot springs was littered with articles of clothing, mostly underclothing, trash and toilet paper.

Beaver dams were not observed on Conundrum Creek or on Castle Creek below Elk Mountain Lodge downstream to near Queens Gulch. Beaver possibly inhabit this stretch of Castle Creek but much of it is on private land and not readily available for examination. In the area below Queens Gulch, 3 of 6 fecal samples from beaver dams were positive for *Giardia*, as were 4 of 26 samples from the Elk Mountain Lodge area. No other animal species were found positive for *Giardia*.

Dog feces was picked up whenever it seemed fresh enough to be a valid sample (not dried out or exposed to freezing temperatures). Feces was found along every trail and campground visited but few were fresh enough to be used. During 1975, 10 samples were obtained from the dog pound in Aspen operated by Lew Lou's Boarding Kennels, and 7 of these contained *Giardia*. In 1977 additional samples were collected consisting of 11 samples from kenneled dogs and 5 from pound dogs. All 11 samples from kenneled dogs were negative and 60% (3 of 5) of the pound dog samples were positive for *Giardia*. All dog feces picked up from trails and campgrounds was negative for *Giardia* although other parasite ova were found.

During the summer of 1976, 130 *Giardia*-sampling packets were given to backpackers going into the Maroon Bells-Snowmass Wilderness Area. Of the packets, 25% (33 of 130) (3 samples from dogs and 30 from humans) were returned. No dogs were infected, but 6.6% (2 of 30) human samples contained *Giardia* (Table 3). The survey was repeated in 1977, but the road to Maroon Lake was closed to all but camper traffic and all other visitors, including backpackers, rode to the lake in buses and a very small number of packets were given out. Of the 12 which were returned, all were negative for *Giardia*.

## Vermejo Park

Samples from two rock squirrels and 10 wapiti taken from Vermejo Park in 1975 were negative for *Giardia*. In 1976, 34 coyote samples were collected from this area and 6% (2 of 34) were infected with *Giardia*. This area is used by very few people and there are no camping areas.

*Table 3 - Results of 1976 human survey for* Giardia *in the Maroon Bells area of Central Colorado.*

| Sample # | Age | Sex | Results | Return City & State |
|---|---|---|---|---|
| 1 | 29 | M | Neg. | Boulder, Colorado |
| 2 | 24 | M | Neg. | -------- |
| 3 | 28 | M | Neg. | Boulder, Colorado |
| 4 | 28 | M | Neg. | Chicago, Illinois |
| 5 | 33 | M | Neg. | Littleton, Colorado |
| 6 | 51 | M | Neg. | -------- |
| 7 | 21 | M | Neg. | -------- |
| 8 | 29 | M | Neg. | San Francisco, California |
| 9 | — | M | Neg. | Denver, Colorado |
| 10 | — | M | Neg. | Fort Morgan, Colorado |
| 11 | 2 | M | Neg. | El Paso, Texas (Dog) |
| 12 | 17 | M | Neg. | Boulder, Colorado |
| 13 | 24 | F | Neg. | Denton, Texas |
| 14 | 25 | F | *Positive* | San Francisco, California |
| 15 | 41 | F | Neg. | Littleton, Colorado |
| 16 | 23 | M | Neg. | Denton, Texas |
| 17 | 28 | M | Neg. | El Paso, Texas |
| 18 | 23 | M | Neg. | Pasadena, California |
| 19 | 28 | F | Neg. | Venice, California |
| 20 | 9 | F | Neg. | Aspen, Colorado |
| 21 | — | — | Neg. | Lakewood, Colorado |
| 22 | 53 | M | Neg. | Lakewood, Colorado |
| 23 | 23 | M | Neg. | Aspen, Colorado |
| 24 | — | M | *Positive* | Portland, Indiana |
| 25 | 8 | M | Neg. | Ft. Collins, Colorado (Dog) |
| 26 | 26 | M | Neg. | Snowmass, Colorado |
| 27 | — | — | Coccidia | Glenwood Springs, Colorado |
| 28 | — | — | Neg. | Glenwood Springs, Colorado (Dog) |
| 29 | 26 | M | Neg. | Snowmass, Colorado |
| 30 | 28 | F | Neg. | Aspen, Colorado |
| 31 | 27 | F | Neg. | Aspen, Colorado |
| 32 | — | — | Neg. | -------- |
| 33 | — | — | Neg. | -------- |

## Rocky Mountain National Park

Following an outbreak of giardiasis in a group of tourists staying at the Cascade Cabins on Fall River within Rocky Mountain National Park, the entire length of Fall River from the location of the water siphon for the Cascade Cabins to its headwaters was examined. Fall River winds its way through a broad meadow for approximately 8 km (5 miles); above this it is a very steep, rapid flowing creek in a narrow canyon originating in a cirque basin. Human use of this entire stretch of river is very heavy. Wildlife in this area is varied and consists of beaver, muskrat, bighorn sheep, mule deer, wapiti, raccoon and coyote. As we have been asked several times if fish could carry *Giardia* we captured 10 brook trout from the pond where water for Cascade Cabins was taken. All were negative. Horses are used infrequently along the river and only one fresh sample was found; it was negative for *Giardia*. One dog sample was found along the river and, even though it appeared to be at least a day old, it was examined. *Giardia* cysts were not found. One human female was observed defecating along Fall River, within 1.5m (5 feet) of the water in a very moist area, in spite of the presence of an outdoor toilet within 185 m (200 yards) which was in plain view and clearly marked with signs. This sample was negative for *Giardia*. Field crews from the Center for Disease Control (CDC) and Environmental Protection Agency (EPA) pumped water from Fall River and did detect *Giardia* cysts in a sample from a beaver pond. *Giardia* was not recovered from beaver feces from this location or upstream from it.

Near the visitor center at the top of Fall River Pass, there is an open sewage plant for the visitor center. The possibility exists that material from this sewage plant could leak into Fall River.

### Brush Creek

Brush Creek is a tributary of the Eagle River and empties into the Eagle River near the town of Eagle. Near Eagle the terrain is fairly flat. As the river is followed upstream towards the Sawatch Mountains the topography becomes more severe and the river valley narrows. The slopes are steep but not as severe as those near Aspen, nor are the ridges as towering. A Colorado resident with a documented case of giardiasis stated the only untreated water which he drank was from East Brush Creek. For this reason samples were collected on East and West Brush Creek. Samples from beaver and cattle were obtained on West Brush Creek. None of 14 beaver and 1 of 11 cattle were infected with *Giardia*. These cattle, about 15-18 animals, were grazing beside West Brush Creek and they were observed defecating into the creek. East Brush Creek flows through a canyon which is a bit more rugged than West Brush Creek. Cattle were not observed in the area but old feces were found along the river. Of 18 beaver fecal samples picked up below Yeoman Park Campground, 17% (3 of 18) were positive for *Giardia*.

East Brush Creek is a fast flowing creek until it reaches the upper portion where it flows through a large meadow. Two campgrounds, Yeoman Park and Fulford Cave, have been established here. Along the river near the Yeoman Park Campground, human feces and toilet paper were found in several locations. This stretch of river is densely vegetated with willow which provides cover for people using the river for a toilet.

### Middle Park

Middle Park is a large mountain basin in north-central Colorado. The Continental Divide forms the park boundary on the north, east, and south sides and the Gore Range on the west. The lowest point in Middle Park is west of Kremmling where the Colorado River flows through Gore Canyon at an elevation of 2200 m (7300 feet). Some of the surrounding mountain peaks are in excess of 3900 m (13000 feet). The major vegetation type varies from sagebrush to spruce-fir and alpine. The entire area is heavily used all year for recreation by residents and many tourists use the area for vacationing. Spring and summer activities are primarily hiking, backpacking, camping and fishing. Hunting is a major activity during the fall as is wood gathering. Hiking, camping and fishing are other major activities, as are snow-mobiling and skiing in winter.

As stated before, backpackers were the first to complain of giardiasis in this area. The first area to be examined was the Vasquez River above Hideaway Park. Several outbreaks in visitors at guest lodges in the area had been documented. Water for the lodges was taken from the Vasquez River. This drainage is part of the Denver water collection system and outbreaks of giardiasis have been documented in resorts using water out of the Denver water siphon. These resorts use chlorinated but unfiltered water only.

The slopes along Vasquez Creek are fairly steep, and usually heavily wooded with aspen, spruce and firs. Very little wildlife was observed along the stream other than a few red squirrels. Higher on the slopes in rocky, dry ground, pika, marmot, golden mantled ground squirrels and chipmunks were found. All samples from these animals were negative for *Giardia*. Beaver or beaver dams were not seen on the Vasquez watershed. One herd of wapiti was found on the ridge north of the west branch of Vasquez Creek. This herd numbered 31 cows and 17 calves. Six fecal samples were picked up and all were negative for *Giardia*. Even though this is a fairly rugged area, humans were seen every day. Feces of humans was not found near any open water. None of the animal feces examined was positive for *Giardia* from these areas.

The area around Hot Sulphur Springs was examined following reports of giardiasis in area residents. *Giardia* cysts were found in 2 of 6 bovines near Corral Creek and 3 of 5 on Cow Creek. As this is dry country, the cattle were very close to the creeks and were observed defecating into both creeks. Richardson's ground squirrels were collected within 30 m (100 feet) of where the positive cattle were grazing, and all 5 samples were negative for *Giardia*.

Since animals positive for *Giardia* were not found at higher elevations, streams at lower elevations were examined. Beaver Creek flows into the Colorado River at the west end of Byers Canyon about 4 km (2.5 miles) southwest of Hot Sulphur Springs. Several campgrounds and summer homes are found along Beaver Creek. The stream originates near Church Park and is heavily utilized by humans. A few cattle and horses are seen occasionally and deer and elk are seen year around at different elevations. Beaver samples collected near the campground where Beaver Creek joins the Colorado were positive for *Giardia*. Samples were then taken from beaver dams all the way up Beaver Creek. Beaver samples from approximately one-third of the dams were positive. All of the positive samples were obtained from an area extending from the Beaver Creek Campground to the private homes.

The following spring none of the beaver samples collected along Beaver Creek was positive but by October, 42% of the samples were positive for

*Giardia*. Of these, 11% (1 of 9) of the samples were positive from above the campground but 52% (14 of 27) at the campground were positive. From Williams Fork River, where a large percentage of miners was supposed to be infected with *Giardia*, 4 beaver samples were negative. Beaver samples also were collected from Willow Creek, which flows along Colorado highway 125. Of the samples collected, 23% (9 of 39) contained *Giardia* cysts. On the upper reaches of Willow Creek none of the samples were positive. The first positive samples came from a beaver pond near the highway and from this location downstream, positive samples were taken from every beaver dam.

Many campgrounds are located along Willow Creek. Wells at Saw Mill Gulch and an unnamed picnic area with hand pumps were declared "unsafe" by the Grand County Environmental Health Department.

Just inside the mouth of Byers Canyon, approximately 400 m (450 yards) west of Hot Sulphur Springs, two streams flow from the side of the road. Samples taken from these springs by the Colorado Dept. of Health showed unsatisfactory coliform results and were positive for *Giardia* cysts.

## RESULTS OF EXPERIMENTAL INFECTIONS

Animals exposed to human-source *Giardia* produced varied results both within groups of animals and among groups of animals (Table 4). Hamsters, domestic rabbits, laboratory mice and deer mice remained uniformly negative. Attempts to infect calf and adult wapiti, fawn and adult mule deer, fawn and adult white-tailed deer, black bear, domestic sheep and cattle have failed. Animals which did become infected and passed *Giardia* cysts were laboratory rats, gerbils, guinea pigs, beaver, dogs, raccoons, bighorn X mouflon sheep and pronghorn. Cysts recovered from these animals ranged in size from 9.5 to 11.0 μm X 8.0 to 9.5 μm. Cysts from pronghorn and naturally infected mule deer were often distorted and were about half as wide as non-deformed cysts.

The first group of rats exposed to human-source *Giardia* was kept in a single cage and composite fecal samples were examined. *Giardia* cysts were found on 22, 25 and 40 days (Table 4) post exposure (PE) and were of 2 sizes, one 5μm long the other 10 μm long, yet identical in all other aspects. The second group of rats was kept in separate cages. One rat shed cysts for 1 day, 34 days PE. All others were negative on fecal examination and at 40 days when the animals were sacrificed, trophozoites were not seen in any intestinal smears. Feces from the infected rat was fed to a beagle pup which became infected and started shedding *Giardia* cysts 8 days PE.

A total of 4 groups of gerbils (each group consisting of 4 exposed animals and 1 in-group control animal) were exposed to human-source *Giardia*. In the first group, 3 of the 4 exposed animals became infected between 13 and 18 days PE (Table 4). Thirty-three days PE the control animal had *Giardia* cysts in the feces. The remaining exposed animal remained negative for 45 days. One gerbil from 2 additional groups was positive for *Giardia* 8 days PE for 1 day. The last group of gerbils remained negative for *Giardia* for 42 days PE. Trophozoites were not seen in necropsied animals. One group of guinea pigs

*Table 4 - Results of experimental infections using human* (Homo sapiens) *source* Giardia.

| Experimental Animal* | Results |
|---|---|
| Hamster *(Mesocricetus auratus)* (4+1) | Negative - 34 days |
| Domestic rabbit *(Oryctolagus cuniculus)* (4+1) | Negative - 28 days |
| Laboratory mice *(Mus musculus)* 4 groups of (4+1) | All negative - 40 days |
| Deer mice *(Peromyscus maniculatus)* 2 groups (4+1) | Negative - 22 days |
| Rats *(Rattus norvegicus)* | |
| (4+1) | Composite fecals - positive - day 22, 25 and 40 (Cysts of 2 sizes) |
| (4+1) | One rat positive 34 days post-exposure for 1 day |
| Gerbils *(Gerbillus gerbillus)* | |
| (4+1) | #1 positive day 22,26-29,32-33<br>#2 positive day 13,19-22,26-28<br>#3 Control-positive day 33-35<br>#4 Positive day 18-21,26<br>#5 Negative 45 days |
| 2 groups (4+1) | One gerbil was positive for 1 day 8 days post-exposure |
| (4+1) | Negative - 42 days |
| Guinea pig *(Cavis porcellus)* (4+1) | One animal became positive on day 21 and remained positive for 31 days. All others were negative. |
| Beaver *(Castor canadensis)* | |
| (2+1) | Negative 38 days. |
| (2+1) | Both positive 25 days after exposure; cysts seen for 22 days |
| Dog *(Canis familiaris)* | |
| (2+1) | Positive 6 days post-exposure for 14 days Animals treated. |
| (2+1) | Positive 8 days post-exposure for 14 days Animals treated. |
| Raccoon *(Procyon lotor)* | |
| (3+1) | Negative - 28 days |
| (3+1) | Negative - 43 days |
| (3+1) | Negative - 34 days |
| (1) | Positive 8 days post-exposure for 1 day - Negative for the next 20. |
| Black bear *(Ursus americanus)* | |
| (1) | Negative - 28 days |

*Table 4 - continuation . . .*

| Experimental Animal* | Results |
|---|---|
| Bighorn X mouflon sheep *(Ovis canadensis* X *O. musimon)* | |
| (4+1) | #1 - Negative<br>#2 - Negative<br>#12 - Control - Negative<br>#29 - Positive 9 days for 4 days<br>#44 - Positive 9 days for 4 days |
| Wapiti *(Cervus canadensis)* | |
| (2+1) | Negative - 30 days |
| (2+1) | Negative - 28 days |
| Mule deer *(Odocoileus hemionus)* | |
| (2+2) | Negative - 28 days |
| (4+2) | Negative - 30 days |
| (3+6) | Negative - 22 days |
| Pronghorn *(Antilocapra americana)* | |
| (1+1) | Positive days 16-18 post-exposure |
| Domestic sheep *(Ovis aries)* | |
| (3+1) | Negative - 45 days |
| Domestic cattle *(Bos tarus)* | |
| (4+1) | Negative - 45 days |
| White-tailed deer *(Odocoileus virginianus)* | |
| (1+2) | Negative - 30 days |
| (1+6) | Negative - 22 days |

**number of infected animals followed by number of in-group controls.*

was exposed to human-source *Giardia* (Table 4). One animal had *Giardia* cysts in the feces 21 days PE and remained positive for 31 days. As each guinea pig was held in a separate cage no chance for transmission between animals existed. The remaining 4 animals were negative 58 days PE.

Three beaver were maintained in captivity and all were negative for over 40 days before 2 were exposed to human-source *Giardia*. The control animal and 1 previously exposed animal were each given 10,000 human source *Giardia* cysts. Both of these animals had *Giardia* cysts in their feces 25 days PE and remained positive for 22 days. Both animals then became negative for 7 to 15 days after which they again were positive. This pattern was followed for approximately 3 months. The control animal remained negative.

Two groups of 2 dogs each were exposed (Table 4) to human-source *Giardia*. In one group both infected dogs became positive at 6 days PE, and the in-group control was positive 7 days later. The second group of infected dogs started shedding *Giardia* cysts 8 days PE and the in-group control became infected 7 days later. In both cases control animals kept in the same room remained negative.

A total of 10 raccoons was infected with *Giardia* from a human source (Table 4). One young raccoon became positive for *Giardia* 8 days PE for 1 day and only 3 cysts were found. One black bear cub (age about 6 months), exposed to human-source *Giardia*, remained negative for 28 days.

Four bighorn X mouflon sheep were given human-source *Giardia*. Two of the 4 plus the in-group control remained negative (Table 4). The other 2 shed *Giardia* cysts from day 9 through 13 PE and both had loose stools during this time. Feces from both animals were collected and given to beagle puppies. The pup given *Giardia* cysts from sheep #29 became positive 10 days PE yet *Giardia* cysts from sheep #44 failed to infect a pup. Control dogs remained negative. One pronghorn fawn (2.5 months old) was positive 16, 17 and 18 days after exposure. He remained negative for an additional 10 days. Control animals remained negative throughout this time.

*Giardia* cysts isolated from beaver feces collected from beaver ponds on Beaver Creek near Hot Sulphur Springs, Colorado were given to laboratory mice, rats, guinea pigs, and hamsters, all of which remained negative (Table 5). Cysts from the same source were given to beagle puppies and, 8 days later, cysts were found in the feces of 4 of the infected pups but not the in-group

*Table 5. Results of experimental infections using Beaver* (Castor canadensis) *source* Giardia.

| Experimental Animal* | Results |
|---|---|
| Mice *(Mus musculus)*<br>2 groups (4+1) | All negative |
| Rats *(Rattus norvegicus)*<br>2 groups (4+1) | All negative |
| Hamsters *(Mesocricetus auratus)*<br>(4+1) | Negative - 30 days |
| Guinea pig *(Cavia porcellus)*<br>(4+1) | Negative - 30 days |
| Dog *(Canis familiaris)*<br>(4+1) | All 4 exposed animals positive 8 days after exposure - control positive 9 days later - allpositive 21 days post-exposure |
| Human *(Homo sapiens)*<br>(3+1) | #1 Positive 11 days post-exposure<br>#2 Positive 6 days post-exposure<br>#3 Negative-subject was on Tetracycline therapy |

**Number of exposed animals followed by number of in-group control animals.*

control. The control animal was positive 9 days after cysts were found in the 4 experimental animals.

Cysts from beaver were also given to 3 human volunteers. One of the volunteers had cysts in his feces 6 days PE, another at 11 days PE. The third volunteer remained negative as did the control. It was later determined the third volunteer was on tetracycline therapy.

*Giardia* cysts isolated from 1 of 4 naturally-infected, captive mule deer fawns were given to 2 beagle puppies, which remained negative, and 1 human volunteer who started shedding *Giardia* cysts in the feces 9 days after exposure (Table 6).

*Table 6 - Results of experimental infections using Mule deer* (Odocoileus hemionus) *source* Giardia.

| Experimental Animal* | Results |
|---|---|
| Dog *(Canis familiaris)*<br>(2+5) | Negative 28 days |
| Human *(Homo sapiens)*<br>(1) | Positive 9 days<br>post-exposure |

* *Number of exposed animals followed by number of in-group controls.*

Cyst output varied greatly in the animals. The number of cysts would be very high one day and then quite low the following day. Dogs and beaver produced the most consistent numbers of cysts.

## DISCUSSION

Giardiasis is a growing problem in Colorado and will probably remain so for many years. An ever increasing number of people and their pets using the outdoors for recreation, lack of sanitary facilities and reluctance to use them, inadequate sewage and water treatment systems are important factors in the epidemiology of sylvatic giardiasis. There is no doubt that animal reservoirs exist and contribute to the problem. However, except for dogs and cats, infected animals were not found in the spring. Beaver and cattle were not found positive for *Giardia* until mid to late summer and fall. This suggests that these animals lose their *Giardia* infections over winter and become reinfected the following summer.

Since prevalence of *Giardia* was high and beaver became infected with human *Giardia*, beaver may be an excellent sentry animal for *Giardia*. One such instance was found on the Fraser River where all beaver fecal samples examined above the sewage treatment plant at Fraser, Colorado, were negative for *Giardia* while beaver directly below the treatment plant were positive. Thus, the sewage plant was probably a source of *Giardia* in the Fraser River.

Beaver are an important reservoir of *Giardia* since they carry the infection for at least 3 months and defecate into the water where *Giardia* cysts survive very well. People often drink water from beaver dams and often collect water as it flows over the dam, not realizing beaver defecate on the other side of the dam.

Cattle tend to congregate around water because much of their grazing range is dry. They often defecate around or in water and also walk through feces getting to the water. For this reason and the fact that *Giardia* was found in them, cattle become important as reservoirs.

Dogs accompany man almost every where he goes including his recreational trips. Dogs also have habits of coprophagy and a desire to roll in feces. These activities increase the animal's chance of becoming infected with *Giardia* and bringing the cysts back to their owners on the haircoat. Dogs have been observed defecating near open streams; people, not wanting to see or step in the feces, proceed to kick the feces into the stream.

Cats are probably not very important in sylvatic giardiasis away from the home. Cats do not travel with backpackers and only rarely with campers. Near home, however, a cat could become infected while out of the house and then return home to infect its owners. Two such instances are known; a family of 5 in Fort Collins had giardiasis and the family cat was shedding large numbers of *Giardia* cysts in a normal appearing stool. A resident of Hideaway Park, Colorado and her boyfriend both had severe giardiasis and, when a stool from the female's cat was examined, large numbers of *Giardia* cysts were found. The question remains as to whether the cats infected the people or the cats obtained the infection from the people.

Humans are the most important component in the epidemiology of giardiasis. The Snowmass-Maroon Bells Wilderness area has been aptly described as "one of the world's largest open air toilets". People have been observed defecating near streams with outdoor toilets in sight. Perhaps they think this activity is a necessary part of the wilderness experience. Diapers containing feces have been found in lakes and streams and there are reports of people emptying recreational vehicle (RV) toilets into rivers. In each study area a common complaint of county officials is the lack of sewage disposal by owners of mountain homes. Instances of direct dumping of sewage into streams have been found. The very limited survey of people going into the Maroon Bells-Snowmass Wilderness Area showed people already infected with *Giardia* are going into the back country. As the trails in the area follow streams, it is easy to visualize how contamination of these streams, which always lead toward a more populated area, can occur. People, especially day hikers, generally are not in physical condition for strenuous hiking and these people drink large amounts of water from the streams near the trails.

Wild ruminants, common inhabitants of mountainous watersheds, were not infected with *Giardia*. Four cases of *Giardia* infection in captive mule deer fawns were found in 1977; all deer had severe diarrhea for over 2 months. These animals were all orphans which were being hand-raised along with other uninfected mule deer. One of the infected fawns died 3 weeks after being treated for *Giardia* and being *Giardia*-free for the 3 weeks. At necropsy a marked absence of thymic tissue was noted. As a result of the thymic atrophy this animal, and most likely the other 3 , was immunologically incompetent and, therefore, susceptible to *Giardia*.

Measurements of cysts recovered from experimentally infected animals were within the limits for *G. lamblia* cysts(6) except for the small cysts which were recovered from the first group of rats infected with human-source *Giardia.* It is possible 2 species of *Giardia* were present, but since trophozoites were not recovered, this question remains unanswered.

In several instances only 1 or 2 animals of a group would shed cysts after infection. This is most likely due to the individual animals immune status at the time of exposure. The immune response may also dictate the invasiveness of the cysts shed by the host. A possible example is the bighorn sheep X mouflon hybrid experiment in which 2 of the 4 infected animals shed cysts and cysts from only 1 of the sheep were infective to beagle pups. The dog which remained negative was later infected with human-source *Giardia* and cysts were found in the feces 9 days after infection. Rats, hamsters, and guinea pigs showed similar results. The host immune response may also be responsible for some of the variability in cyst production which was observed in infected animals.

It is possible that some of the animals were infected but, because they were shedding only small numbers of cysts which were not recovered with the $ZnSO_4$ method, they were termed negative. However, *Giardia* cysts or trophozoites were not recovered at necropsy from animals negative for *Giardia* cysts using the $ZnSO_4$ method.

It appears that human-source *Giardia* will not infect hamsters, laboratory mice, deer mice, mule deer, wapiti or white-tailed deer. Inconsistent infections resulted in rats, gerbils, beaver, guinea pigs, bighorn X mouflon sheep and raccoons given human-source *Giardia* cysts. Additional infections of domestic sheep and cattle, pronghorns and black bear must be completed before definite conclusions are made concerning their ability to become infected with human-source *Giardia.*

In most cases (pronghorn, raccoon, rats and bighorn X mouflon sheep) the patent period lasted only a few days. Gerbils had a longer patent period but cysts were not detected every day (Table 4). The longest patent periods were observed in beaver (at least 22 days) and beagle pups (at least 3 months). Shedding of cysts by beaver became somewhat cyclic after 22 days, possibly reflecting reinfection or a true latent period. Dogs were monitored for *Giardia* cyst production and while the number of cysts shed varied greatly, the animals remained infected for over 3 months (unpublished data).

Infection of animals with *Giardia* cysts from beaver paralleled the results of human-source *Giardia* in that mice and hamsters did not become infected. Guinea pigs and rats did not become infected which was unlike the results obtained using human-source *Giardia.* Beagle pups did become infected and continued to shed cysts for 21 days when the dogs were terminated. In humans 2 of 3 also became infected after ingesting beaver-source *Giardia* cysts. The third person was on a regimen of 250 mg tetracycline 3 times per day for a non-related infection. Tetracycline will alter the intestinal pH and may have prevented establishment of *Giardia.*

The results from infections with mule deer-source *Giardia* are confusing. It is not known why these cysts resulted in infection of a human, but not in beagle pups which were successfully infected with human-source *Giardia* 30 days later.

Human-source *Giardia* displayed a remarkable shelf life as long as the cysts were cleaned and stored in distilled water in a refrigerator. Cysts were stored for as long as 3 weeks and still resulted in patent infections in dogs. It is not recommended that cysts be held this long as their internal structures do break down.

Cysts which were distorted were recovered from mule deer with a natural infection and a pronghorn with a human-source infection. The distortion consisted of a constriction of the cyst wall and the trophozoites pulling away from the cyst wall. When the feces from the animals was soft or diarrheic the cysts were not distorted but as the feces became formed and dryer distorted cysts were found.

This study has presented prevalence data for a variety of domestic and wild animal hosts which have not previously been studied for *Giardia* in Colorado. Evidence is also provided that beaver and cattle are reservoirs for sylvatic giardiasis in Colorado.

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## APPENDIX A.
## SCIENTIFIC NAMES(25) OF ANIMALS LISTED IN TABLES 1 AND 2.

| | |
|---|---|
| Badger | *Taxidea taxis* |
| Beaver | *Castor canadensis* |
| Bighorn sheep | *Ovis canadensis* |
| Black bear | *Ursus americana* |
| Bobcat | *Lynx rufus* |
| Bovine | *Bos taurus* |
| Brook trout | *Salvelinus fontinalis* |
| Colorado chipmunk | *Eutamias quadrivittatus* |
| Cottontail rabbit | *Sylvilagus* spp |
| Coyote | *Canis latrans* |
| Dog | *Canis familiaris* |
| Domestic sheep | *Ovis aries* |
| European ferret | *Mustela putorius* |
| Golden-mantled ground squirrel | *Spermophilis lateralis* |
| Horse | *Equus caballus* |
| Human | *Homo sapiens* |
| Marmot | *Marmota flaviventris* |
| Mountain lion | *Felis concolor* |
| Mule deer | *Odocoileus hemionus* |
| Muskrat | *Ondatra zibethica* |
| Pika | *Ochotona princeps* |
| Pine marten | *Martes americana* |
| Porcupine | *Erethizon dorsatum* |
| Raccoon | *Procyon lotor* |
| Red fox | *Vulpes fulva* |
| Red squirrel | *Tamiasciurus hudsonicus* |
| Richardson's ground squirrel | *Spermophilus richardsoni* |
| Rock squirrel | *Spermophilus variegatus* |
| Striped skunk | *Mephitis mephitis* |
| Wapiti | *Cervus canadensis* |
| White-tailed deer | *Odocoileus virginianus* |
| White-tailed jackrabbit | *Lepus townsendii* |
| White-tailed prairie dog | *Cynomes leucurus* |

## Discussion

R. FREEMAN: The uniqueness of the mountain situation you referred to intrigues me. For some 20 years I have worked in Canada. We certainly have a tremendous amount of water routes which people follow and I am sure they are drinking untreated water just as much out of the streams, as you have experienced people drinking water in the mountains. What is so unique about your situation that would not apply elsewhere in North America? I am sure our physicians are equally qualified in diagnosing and if this condition were particular to certain areas only, physicians would be informed very quickly.

Could it be that there is not enough sedimenting in areas with a great deal of flowing water? We have flowing water in Canada, but most of our rivers are short, ending in a deep lake where there is a lot of sedimenting. We certainly have very thin soil, so it is not the same situation you have in Colorado. However, due to the influx of Americans into Canada, I am sure we have as much *Giardia* cyst potential. I do not understand the difference in the number of cases found in your studies compared to those in Canada.

R. DAVIES: I have no idea what is so unique unless we have a great deal more travelers who report their infections. Bill Samuel from the University of Edmonton, says that *Giardia* cases around there are extremely high, and most of his graduate students have developed *Giardia* infections. Possibly giardiasis is being examined more closely in Colorado than anywhere else and thus the numerous outbreaks of giardiasis appear to be unique.

L. McCABE: I would think the Canadian situation might not be unique. I am told that the International Nickel Company lowered the pH to 3.5 in the lakes receiving fallout from their stacks.

SPEAKER: Mr. Davies, would you please reiterate the material shown on the last slides.

R. DAVIES: We took *Giardia* that we isolated from beaver found in the Beaver Creek area. This was given to 4 beagle pups and they developed the disease. We also exposed 3 humans. One became positive at 6 days post-inoculation and 1 at 11 days post-inoculation. The third remained negative, but he was on tetracycline therapy, which I have been informed can alter the pH of the gut. This possibly prevented *Giardia* from becoming established.

D. JURANEK: How well screened were the individuals who were taking *Giardia* cysts?

R. DAVIES: Since we have been working with *Giardia*, periodically we all check ourselves to determine if we have become infected and are carriers. Stool samples taken before our experiments began were negative, with the exception of that from Dr. Hibler who had a previous *Giardia* infection. He did become positive again.

J. HOFF: Were the cysts recovered from these various animals morphologically similar to *lamblia*, or were there different morphological types of cysts?

R. DAVIES: All of the cysts we have recovered from wild, free ranging mammals have been morphologically similar, if not exact, to *lamblia*, and the cysts all fall within the limits of those published for *G. lamblia*, including everything obtained from the experimental infections.

W. JAKUBOWSKI: Have you determined whether or not there is a difference in the percent of positive animals in areas receiving a lot of human use as opposed to those areas receiving very little or no human use?

R. DAVIES: That is difficult to say. The areas that we examined were chosen because of the heavy human use and the high risk in those areas. The places where we did find animals which were not infected were along the upper Colorado River. There is a lot of use by people who primarily are just taking a little hike down to the river and back. Backpackers do not use this area. I was surprised that we did not find anything positive there or up the Williams Fork, an area used by many miners.

# Waterborne Outbreaks of Giardiasis

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## ABSTRACT

Data are presented on waterborne outbreaks of giardiasis that have affected travelers to foreign countries, particularly Americans to the Soviet Union, and that have been documented in the United States, including 2 retrospective epidemiologic studies of endemic giardiasis associated with consumption of untreated water.

During the past 5-year period, the most commonly identified pathogen in waterborne outbreaks in the United States has been *G. lamblia*. Twenty-three waterborne outbreaks of giardiasis affecting 7,009 persons have been reported in the United States since the outbreak in Aspen, Colorado in 1965-1966. These have occurred primarily in mountainous areas of this country particularly in New England, the Pacific Northwest, and the Rocky Mountains and have generally involved small municipal water systems or semi-public water systems in recreational areas. Most of the outbreaks occurred as the result of consuming untreated surface water or surface water treated only with chlorine.

The data from waterborne outbreaks of giardiasis in the United States indicate that simple disinfection as the only treatment for surface water sources is ineffective in preventing waterborne transmission of this infection and that to protect against transmission, all surface water should receive pretreatment, preferably with sedimentation, and filtration in addition to chlorination.

The data also indicate that negative results of coliform tests do not provide assurance that the water is free of *Giardia* cysts. In several outbreaks where disinfection was provided, *Giardia* cysts were found in the absence of coliforms. Since disinfection practices in surface water systems where chlorination is the only treatment do not generally provide for high concentrations of chlorine or long contact time, it is likely that *Giardia* cysts would survive treatment whereas coliforms would not.

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*Giardia lamblia* is a flagellated protozoan of the small intestine. In the past its pathogenicity has been questioned; however, there is increasing clinical and epidemiologic evidence that *G. lamblia* is a cause of acute illness and a significant pathogen of man. Clinical manifestations of *Giardia* infection can range from asymptomatic cyst passage to severe malabsorption. Prominent symptoms include diarrhea, abdominal cramps, fatigue, weight loss, flatulence, anorexia, and nausea. An incubation period of 1 to 8 weeks is typical, and the mean duration of acute illness is often 2 to 3 months(1-4).

Giardiasis is endemic in the United States and in many other countries of the world; however, only recently has *G. lamblia* been recognized as the etiologic agent in several common source outbreaks. Although many questions remain to be answered about this infection, there is much that has been learned in recent years through epidemiologic studies of the endemic and epidemic occurrence of giardiasis.

This report will review the data on waterborne outbreaks of giardiasis that have affected travelers to foreign countries, particularly Americans to the Soviet Union, and that have been documented in the United States, including 2 retrospective epidemiologic studies of endemic giardiasis associated with consumption of untreated water.

In discussing an outbreak of amebiasis that affected 150 American occupants of a Tokyo apartment building during December 1946 to January 1947, Davis and Ritchie(5) provided some early epidemiologic evidence of the waterborne transmission of *G. lamblia*. The outbreak was attributed to sewage contamination of the building's water supply, and *Entamoeba histolytica* was recovered from 96 (64%) of the occupants and *G. lamblia* from 116 (77%). There were 26 occupants who experienced diarrhea with abdominal discomfort but whose stools were found to be negative for *E. histolytica*. *G. lamblia* was found in the stools of 20 (80%) individuals in this group suggesting that this organism may have been responsible for some of the illness in this outbreak.

## WATERBORNE GIARDIASIS IN TRAVELERS

The first reports of epidemic giardiasis among travelers to the Soviet Union appeared in 1970 when 2 outbreaks affecting American travelers were investigated by the Center for Disease Control (CDC)(6, 7). Twenty-three of 80 (29%) persons who had accompanied the U.S. Olympic Boxing Team on a tour of the Soviet Union in February and March 1970 and 84 of 164 (51%) scientists visiting in May 1970 had become ill with giardiasis. *G. lamblia* was found in fecal specimens from 9 patients and 2 asymptomatic persons in the first group and in 23 patients and 3 asymptomatic persons in the second group. The illness usually began toward the end of the trip or shortly after return to the United States, and the principal symptoms included nausea, abdominal cramps, diarrhea with stools that were frequently greasy and malodorous, flatulence, anorexia, and fatigue. Retrospective epidemiologic study of these outbreaks implicated Leningrad as the site of acquisition of the infection and tap water as the probable mode of transmission.

Reports of epidemic giardiasis among American and other tourists to Leningrad steadily increased after these 2 outbreaks, and the resulting retrospective and prospective epidemiologic studies have confirmed the relationship between epidemic giardiasis in tourists and the consumption of tap water in Leningrad(8-19).

The CDC collected information by questionnaire on 1,419 members of 47 individual tour groups to the Soviet Union from 1969-1973(8, 15). Data were obtained from tour groups who came to the attention of CDC because of cases of giardiasis and from lists of tour members provided by travel agents specializing in travel to the Soviet Union. Each traveler submitted information concerning age, sex, illness, symptoms, place of lodging, cities visited, and exposure to food and water. Questionnaires showed that 502 (36%) of these travelers were ill during the tour or shortly after their return to the United States. Defining a case of giardiasis as a positive stool examination and/or a diarrheal illness lasting for 1 week or longer, 324 of the 1,419 (23%) travelers were counted as cases. Rates of attack of giardiasis according to cities visited showed Leningrad to be the only city implicated. No specific hotel in Leningrad could be related to increased risk of infection. Histories of tap water consumption implicated drinking water in Leningrad as the likely source of infection. Of 1,082 persons who drank tap water, 274 (25%) developed giardiasis; of 297 who did not drink tap water, 44 (15%) became infected. In 1 tour group of 400 where tap water consumption was quantitated, risk of infection increased with the amount of tap water consumed per day(8). Other food items such as ice cream, uncooked vegetables, and fruit were not statistically related to an increased risk of infection.

Epidemiologic study of another outbreak of giardiasis affecting 72 of 179 (40%) American travelers to the Soviet Union in March 1975 where tap water was found to be significantly related to acquiring the infection also found a positive correlation between the amount of water consumed and the likelihood of becoming infected(18).

A prospective study of 96 staff members of the National Aeronautics and Space Administration (NASA) who traveled to the Soviet Union in 1973 was also conducted by CDC(8). Stool samples were examined for ova and parasites before the tours left the United States and after their return. None of the 96 NASA members had a *Giardia*-positive stool specimen before travel to the Soviet Union. Twenty-one individuals traveled to Leningrad and 17 (90%) reported illness; 75 individuals did not travel to Leningrad and only 3 (4%) reported illness. Stool examinations after return to the United States showed that 15 of the 17 (88%) individuals who traveled to Leningrad and reported illness were positive for *G. lamblia*.

Another prospective study of travelers to the Soviet Union was conducted by Jokipii and Jokipii(17) and included 60 students from Finland who visited Leningrad in 3 separate tours in 1971 and 1973. Each group stayed at a different hotel while in Leningrad. Stool specimens were examined 1 to 2 days before departure and upon return to Finland. All reported on use of tap water and symptoms of illness. *Giardia* cysts were recovered from 2 students prior to the trip. Both of the students had visited Leningrad within the past year. Fifteen of 43 (35%) individuals who were negative prior to the trip had stool specimens which were positive for *Giardia* cysts after their return from Leningrad. The recovery of *G. lamblia* from stools correlated with the occurrence of symptoms and with the consumption of tap water in Leningrad.

Giardiasis had not previously been reported in residents of Leningrad; however, limited reports of giardiasis in Leningrad residents are now available (see Wolfe, these *Proceedings*). It is difficult to assess if this is a widespread problem among residents, and the question remains as to why many tourists become infected through consumption of tap water while residents apparently do not. If this is indeed the case, it has been suggested that 1 or more of these possibilities might be responsible:

1. Water supplies for various hotels in Leningrad may be separated from the supplies for residents.

2. Giardiasis may not be considered by local physicians in the differential diagnosis of chronic diarrhea among residents.
3. There may be an acquired immunity among Leningrad residents as a result of repeated exposure over the years.

Although epidemiologic evidence has clearly implicated Leningrad as the site of infection and tap water as the probable vehicle of transmission of giardiasis to tourists, it has not been possible to obtain data directly on water supplies and treatment or water samples from Leningrad. There is 1 report in the literature, however, that does offer limited information on the water source and treatment for parts of Leningrad. Ryan and Grainge(20) participated in an international conference of sanitary engineers held in the Soviet Union and were able to visit 1 of the 5 water-treatment plants serving the city of Leningrad. The Neva River is used as the source of water and the raw water is reported to be of relatively good physical, chemical, and bacterial quality. As reported by Ryan and Grainge in mg/l, the turbidity of the raw water is generally 3-7 mg/l except during spring floods when it is as high as 70 mg/l. The bacteriological quality of the raw water ranges from 1 to 10 coliforms per liter. Apparently, because the raw water quality is so good, many industries draw water directly from the river without treatment. It is not known if the various hotels use river water directly, but it is suspected that they do not because of the availability of several water-treatment plants in the city. The water-treatment plant visited had 2 separate sections. One was a conventional system and was not discussed. The second treatment process was described as follows: prechlorination of 1.6 mg/l, screening, rapid sand filtration, aeration for 6-8 min, ammonia feed of 1.2 to 1.6 mg/l, alum feed, rapid mixing, settling, and fluoridation. Upward-flow filtration rather than gravity filtration was utilized because the upward filtration results in longer filter runs with colder water temperatures. For gravity filters, it was found that a filtration rate of 7 to 8 m/h could be maintained when the water was 20° C but only 4 m/h at 1° C. With upward filtration the 7 to 8 m/h could be maintained regardless of water temperature. The sand in the filters is 2 m thick and of 0.5 to 2.0 mm sizes. It was reported that the treated water contains a turbidity of 1.6 mg/l and that coliforms in the distribution system average 1/l. Although this is an incomplete description of the water treatment, it does provide some basis for questioning the adequacy of treatment and operation to protect against the waterborne transmission of giardiasis. Effective disinfection of a water supply with chlorine depends on the type of chlorine residual, the factors of contact time, pH, water temperature, and the presence of suspended material(21, 22). The application of chlorine and ammonia, as practiced in this treatment plant, produces chloramines or a combined available chlorine rather than a free available chlorine for disinfection. Combined available chlorine forms are less effective disinfectants. About 25 times as much combined available residual chlorine is necessary to obtain equivalent bacterial kills as free available residual chlorine under similar conditions of pH, water temperature, and contact time; about 100 times longer contact time is required to obtain equivalent bacterial kills for similar concentrations at the same pH and water temperature. It is significant to note that minimum chlorine requirements for destruction of *E. histolytica* cysts are greater than for bacterial inactivation and that diminished effect at a reduced water temperature is more pronounced even under the best conditions of operation. For example, the minimum recommended cysticidal free available chlorine residual(22) after 30 min contact time at pH 7 for a water temperature of 22 to 25°C is 3 mg/l compared to 10 mg/l under similar conditions at 2 to 5°C. Although coliform levels are reportedly low in the Leningrad water distribution system, it is certainly possible that sufficient contact times and/or concentrations of combined available chlorine are not being provided to inactivate *Giardia* cysts, especially in the cold water temperatures encountered during winter and early spring. The comparision of turbidity values in raw and treated water indicate poor operation of the filtration process possibly related to inadequate control of chemical pretreatment of the raw water or the high filtration rates reported for the upflow filters. This suggests that the filtration provided at certain times would not be effective in removal of *Giardia* cysts.

While Leningrad is one location where there seems to be a continuing risk of travelers acquiring giardiasis through drinking water, sporadic waterborne outbreaks of giardiasis can occur elsewhere in the world. An example occurred in October 1976 when reports of a high incidence of diarrhea in a group of 1,400 Americans who had vacationed at the Portuguese island of Madeira were received upon their return to the United States(23). A mail questionnaire survey suggested waterborne giardiasis as the etiology of the outbreak. Of 859 respondents, 27% developed an illness resembling giardiasis, that is, diarrhea of 1 week duration or longer or diarrhea of shorter duration but accompanied by abdominal distention. *G. lamblia* was recovered from 27 of 58 (47%) ill patients who had a stool examination for parasites; *E. histolytica* was isolated from 3 (5%) persons. Drinking tap water was statistically associated with illness as was consumption of ice cream and raw vegetables; however, the illness was felt to have been most likely acquired through consumption of tap water because contamination of ice cream and raw vegetables by tap water could not be ruled out. It was reported that the drinking water was chlorinated, but additional information on water source and other treatment was not available. A follow-up survey of 90 Americans traveling there in the Spring of 1977 showed only 4.5% developing an illness compatible with giardiasis suggesting that the October outbreak was an isolated event rather than a reflection of an ongoing problem.

## ENDEMIC WATERBORNE GIARDIASIS IN THE UNITED STATES

Giardiasis occurs in both epidemic and endemic forms in the United States, and studies in Colorado and Minnesota suggest consumption of untreated drinking water can be an important cause of endemic infection.

Parasitologic surveys have shown *G. lamblia* to be the most frequently identified parasite in Colorado. For example, a retrospective laboratory survey conducted in 1973 in Colorado showed *G. lamblia* to be present in

40% of all positive examinations for ova and parasites(24). The majority of infected residents had experienced an episode of chronic watery diarrhea with a median duration of 3.8 weeks, bloating, flatulence, and weight loss averaging 5.1 kg. A statewide telephone survey was also conducted of 256 infected residents identified from these laboratory records and 256 controls matched by age, sex, race, and place of residence. An analysis of the data obtained showed: (a) an increased incidence of giardiasis in persons between the ages of 16 and 45 with males and females equally affected; and (b) a higher proportion of cases than controls who visited Colorado mountains (69% vs 47%), camped out overnight (38% vs 18%), and drank untreated mountain water (50% vs 17%). Person-to-person transmission was found to be relatively infrequent and the association between giardiasis and drinking untreated mountain water was strengthened by the fact that other potential sources of infection such as home water sources, swimming in unchlorinated pools, domestic animal exposure, or out-of-state or foreign travel were not associated with *G. lamblia* infections. Bacteriologic examination of water samples from 16 mountain streams located in areas with no permanent human habitation demonstrated fecal coliform contamination in each stream; the highest concentration occurring between June and August (475 to 500 fecal coliforms/100 ml), a seasonal pattern which correlated with the monthly distribution of *G. lamblia* infections. The investigators concluded that giardiasis was endemic in Colorado and that drinking untreated mountain water was an important cause of endemic infection.

A similar but smaller scale study conducted in Minnesota also suggested that consumption of untreated water was an important factor in the endemic occurrence of giardiasis in that state(25). Interviews of individuals identified from state laboratory records as having stool examinations positive for *G. lamblia* during January 1974 to February 1975, showed that 78 had no history of recent foreign travel. Among this group of individuals without foreign travel, it was found that 63% had consumed untreated water during the period of study and 46% had not been out of Minnesota in the 2 months before onset of symptoms. Although these data are interesting, this particular study suffers from the lack of an appropriate control group and no firm conclusions can be drawn.

## WATERBORNE OUTBREAKS OF GIARDIASIS IN THE UNITED STATES

A cooperative effort between the Health Effects Research Laboratory, Environmental Protection Agency (EPA), in Cincinnati, Ohio and the CDC in Atlanta, Georgia, to investigate, document, and report waterborne disease outbreaks in the United States has been in existence since 1971. Local and state health departments investigate waterborne disease outbreaks and at times request assistance from CDC and the EPA. As part of the reporting system, state epidemiologists and engineers in state water supply surveillance agencies cooperate in providing data on waterborne outbreaks to EPA and CDC annually. Data reported to EPA and CDC on waterborne outbreaks of giardiasis are summarized periodically(26-28).

The waterborne outbreaks of giardiasis reported in Table 1 are those in which drinking water has been implicated epidemiologically as the vehicle of transmission of the illness. Only outbreaks associated with water used for drinking or domestic purposes are included. To be considered an outbreak, at least 2 cases of giardiasis must be reported before a common source can be noted and investigated. Single cases of giardiasis reported in backpackers, which may be related to drinking untreated water, have not been included as outbreaks.

In a few of the outbreaks, heavy bacterial contamination of the water or an obvious human source of contamination was found. However, in most of the outbreaks, little or no bacterial contamination of the water was reported. There have been only a few outbreaks where *Giardia* cysts have been isolated from drinking water (Table 2). Brady and Wolfe(29) reported 5 cases of giardiasis in 1973 that were epidemiologically associated with drinking water from an underground cistern on a Tennessee farm. The cistern was apparently contaminated by seepage from a pit privy, but no coliform data were available The association of illness with consumption of water from the cistern was strengthened by the reported finding of trophozoites in water samples taken from the cistern, but the methods used to isolate and identify the trophozoites were not explicitly described. These findings have been questioned by Rendtorff(30) and Wright(31). Rendtorff felt that the investigators either identified *Giardia* cysts or, more likely, trophozoites of another free-living flagellate mistaken for *G. lamblia*. Wright noted that *Giardia* trophozoites, unlike the cysts, are fragile organisms whose survival outside the gastrointestinal tract has not been shown. The first waterborne outbreak of giardiasis where a *G. lamblia* cyst was found in the municipal water supply occurred in Rome, New York, during November 1974 to June 1975(32). A single *Giardia* cyst was found upon microscopic examination of water sediments representing 1,059,800 l of water collected during early May 1975 from the raw water intake. This was also the first time that water from an outbreak had been shown to infect laboratory animals, as giardiasis was produced in specific pathogen-free dogs fed sediment samples collected from the raw water intake. *Giardia* cysts were also isolated from drinking water in the Camas, Washington, and Berlin, New Hampshire, outbreaks and these are discussed in more detail later(33-35). An outbreak of giardiasis near Estes Park, Colorado, in the Rocky Mountain National Park in June 1976 initially affected 9 of 17 persons attending a reunion(36, 37). The group had stayed at summer cabins supplied by water from a small reservoir on the Fall River; the water was chlorinated but not filtered. The outbreak prompted additional studies of 48 other visitors who had stayed at the same cabins during June 1 to July 28 and a control group of 42 who had stayed during the same period at a nearby lodge which received filtered, chlorinated water from a municipal supply. Symptoms of giardiasis were reported in 37% of those in the first group but in none of the controls. *Giardia* cysts were found in a water filtrate sample taken from a beaver pond located upstream from the water supply reservoir, but 11 water samples collected from upstream water sources revealed no fecal coliform counts above 0.5/100 ml.

*Table 1. Waterborne outbreaks of giardiasis in United States*

| Location | Date | Cases | Water Supply | Deficiency | Bacterial Quality of Water |
|---|---|---|---|---|---|
| Aspen, Colorado | Dec. 1965-Jan. 1966 | 123 | M | Sewage contamination of wells; source of water was wells and surface supply with disinfection of each source | Intermittent coliform contamination of water system distribution system |
| Lookout Mt., CO. | Aug. 1969 | 19 | SP | Unknown | — |
| Idyllwild, CA. | May-June 1970 | 34 | M | Surface water source with filtration and disinfection; filters used intermittently | One sample during outbreak showed >16 coliforms/100 ml; 7 samples in 6 months prior to outbreak and 5 samples in July 1970 were all negative; all distribution system samples |
| Campground; Boulder Co., CO. | May 1972 | 28 | SP | Surface water with disinfection only; defective chlorinator | — |
| Resort; High Co., CO. | May 1972 | 24 | SP | Surface water with disinfection only; defective chlorinator | — |
| Camp; San Juan Area, Utah | Sept. 1972 | 60 | SP | Use of untreated surface water | — |
| Subdivision; Park Co., CO. | Dec. 1972-Jan. 1973 | 12 | M | Septic tank seepage into wells; no treatment | — |
| Lodge; Grand Co., CO. | July 1973 | 16 | SP | Use of untreated surface water | — |
| Farm; Tennessee | Aug. 1973 | 5 | I | Seepage from pit privy contaminated cistern | — |
| Essex Center, VT. | Nov. 1973-April 1974 | 32 | M | Surface water with disinfection only | Raw water samples showed source to be contaminated; low coliform count after chlorination |

*Table 1. Waterborne outbreaks of giardiasis in United States (continued)*

| Location | Date | Cases | Water Supply | Deficiency | Bacterial Quality of Water |
|---|---|---|---|---|---|
| Danville Green, VT. | Dec. 1973-April 1974 | 20 | M | Surface water with disinfection only | Watershed survey revealed two homes with septic tank drainage into pond; no chlorine residual in distribution system |
| Lodge; Grand Co., CO. | June 1974 | 18 | SP | Surface water with disinfection only | — |
| Meriden, N.H. | June-Aug. 1974 | 78 | M | Surface water with disinfection only | — |
| Campers; Utah | Sept. 1974 | 34 | I | Use of untreated surface water | Stream samples had mean fecal coliform count of 42/100 ml |
| Rome, New York | Nov. 1974-June 1975 | 4800 | M | Surface water with disinfection only | Routine sampling of distribution system showed coliform levels to be less than 1/100 ml on 527 samples collected from Nov.-June; 4 samples in Feb. showed 20, 20, 30, 40 coliforms/100 ml |
| Campers; Idaho | Sept. 1975 | 9 | I | Use of untreated surface water | — |
| Office & residence; Grand Co., CO. | Feb. 1976 | 12 | SP | Use of untreated surface water | 23 coliforms/100 ml |
| Camas, Washington | May 1976 | 600 | M | Wells and surface water treated by pressure filtration and disinfection; deficiencies found in operation of filters and chlorinators | Routine samples showed finished water to meet coliform standards |
| Camp; Estes Park, CO. | June 1976 | 27 | SP | Surface water with disinfection only | Water source contained less than 0.5 fecal coliforms/100ml |

*Table 1. Waterborne outbreaks of giardiasis in United States (continued)*

| Location | Date | Cases | Water Supply | Deficiency | Bacterial Quality of Water |
|---|---|---|---|---|---|
| Berlin, N.H. | April 1977 | 750 | M | Surface water with conventional treatment and surface water with pressure filtration and disinfection; deficiencies found in construction of gravity filter and operation of pressure filter | Routine samples of distribution system were consistently negative |
| Campground, Utah | June 1977 | 7 | SP | Use of untreated well water influenced by surface water | — |
| Hotel; Glacier Park, MT. | July 1977 | 55 | SP | Use of untreated surface water | — |
| W. Sulfur Springs, Montana | July 1977 | 246 | M | Surface water with disinfection only | — |

*Table 2. Waterborne outbreaks of giardiasis in the United States where* Giardia *have been isolated from water*

| Year | Location | Description |
|---|---|---|
| 1973 | Tennessee | Numerous trophozoites of *G. lamblia* were found in water samples collected from an underground cistern. |
| 1974 | Rome, New York | A single *Giardia* cyst was found after filtering 1,059,800 liters of raw water from the plant intake. |
| 1976 | Camas, Washington | *Giardia* cysts were found in the raw water and distribution system. |
| 1976 | Camp, Estes Park, Colorado | *Giardia* cysts were found in a water filtrate sample taken from a beaver pond located upstream from the water supply reservoir. |
| 1977 | Berlin, New Hampshire | *Giardia* cysts were recovered from both raw water sources and several sites within the distribution system. |

Twenty-three waterborne outbreaks of giardiasis affecting 7,009 persons have been reported in the United States (Table 3). The first waterborne outbreak documented in the United States occurred at Aspen, Colorado(38), during December 1965 through January 1966. The outbreak came to the attention of CDC when a physician at the Center developed characteristic symptoms of giardiasis after return from a ski holiday at Aspen. His stools contained no bacterial pathogens, but cysts of *G. lamblia* were abundant. A survey of 1,094 skiers who had vacationed in Aspen during the 1965-1966 ski season showed that at least 123 (11%) had developed similar symptoms. The association of *G. lamblia* with the illness, the absence of other pathogens, and the response to treatment suggested that *G. lamblia* was the agent responsible for illness.

Approximately half the city's water came from a distant mountain creek serving the western side of the city and half from 3 wells serving the eastern side. Chlorinators were provided at each water source, but coliform contamination of the water system was noted intermittently during the 1965-1966 ski season. Examination of the sparsely populated creek area revealed no obvious possibility of sewage contamination; however, fluorescent and detergent tracers placed in the sewage system were detected in 2 of the 3 wells. Engineering evaluation suggested sewage contamination of the wells from leaking sewer mains near the wells. *G. lamblia* cysts were isolated from sewage in the leaking sewer lines. A parasitologic survey of Aspen residents showed a difference in prevalence of *G. lamblia* infection between the eastern section (6.9%) and the western section (3.7%) but this was not statistically significant. In retrospect, it is possible that the surface water source was also

*Table 3. Waterborne outbreaks of giardiasis in United States*

| Year | Outbreaks | Cases |
|---|---|---|
| 1965 | 1 | 123 |
| 1966 | — | — |
| 1967 | — | — |
| 1968 | — | — |
| 1969 | 1 | 19 |
| 1970 | 1 | 34 |
| 1971 | — | — |
| 1972 | 4 | 124 |
| 1973 | 4 | 73 |
| 1974 | 4 | 4,930 |
| 1975 | 1 | 9 |
| 1976 | 3 | 639 |
| 1977 | 4 | 1,058 |
| Total | 23 | 7,009 |

contaminated with *G. lamblia*; however, this was not sufficiently evaluated at the time.

There has been a steady increase in waterborne outbreaks of giardiasis in the United States since 1971. Increased recognition of this disease because of reports of giardiasis in travelers to the Soviet Union has no doubt been responsible for increased surveillance, investigation, and reporting by public health authorities in the United States.

Waterborne outbreaks of giardiasis have occurred primarily in mountainous areas of this country particularly in New England, the Pacific Northwest, and the Rocky Mountains (Table 4). These areas have traditionally depended upon surface water sources relatively free of human sewage contamination and of good bacterial quality, and treatment has been minimal consisting primarily of disinfection only. Attention to maintaining continuous disinfection has not been as great as in other areas of the country where streams are known to be contaminated with human waste discharges.

Colorado has experienced more outbreaks than any other state and this probably reflects increased surveillance and investigation. Attention focused on Colorado because of the Aspen outbreak, endemic giardiasis, and reports of giardiasis in backpackers has no doubt been responsible for increased surveillance activities.

During the past 5-year period, the most commonly identified pathogen in waterborne outbreaks in the United States was *G. lamblia* (Table 5). There were 20 waterborne outbreaks and 6,833 cases of gardiasis during 1972-1977. It is possible that some of the 100 outbreaks and 16,504 cases of acute gastrointestinal illness of undetermined etiology were giardiasis; however,

*Table 4. Location of waterborne outbreaks of giardiasis in United States*

| State | Outbreaks |
|---|---|
| Colorado | 9 |
| Utah | 3 |
| Montana | 2 |
| New Hampshire | 2 |
| Vermont | 2 |
| California | 1 |
| Idaho | 1 |
| New York | 1 |
| Washington | 1 |
| Tennessee | 1 |

this is unlikely because the symptoms, incubation period, and self-limiting nature of the illness in those outbreaks were not characteristic of giardiasis.

Comparing waterborne outbreaks of giardiasis to other waterborne outbreaks shows the relative importance of this organism as a waterborne pathogen (Table 6). In some years there have been relatively few cases of waterborne giardiasis compared to other waterborne illness, but in other years the number has been quite high. For instance, in 1974 and 1977, *G. lamblia* was responsible for 59% and 27% respectively of the waterborne illness. The Rome, New York outbreak accounted for most of the cases in 1974, and several large outbreaks occurred in 1977.

Waterborne outbreaks of giardiasis have generally affected small municipal water systems or semi-public water systems in recreational areas (Table 7). Municipal water systems are defined as public or investor-owned water supplies that serve communities. Semi-public water systems, located in areas not served by municipal systems, are those developed and maintained at locations where the general public has access to drinking water provided by industries, institutions, camps, parks, hotels, etc. Individual water systems are those used by single residences in areas without

*Table 5. Etiology of waterborne outbreaks in United States, 1972-1977*

| | Outbreaks | Cases |
|---|---|---|
| Acute Gastrointestinal Illness | 100 | 16,504 |
| Chemical Poisoning | 22 | 508 |
| Giardiasis | 20 | 6,833 |
| Shigellosis | 14 | 4,652 |
| Hepatitis A | 9 | 234 |
| Salmonellosis | 5 | 1,000 |
| Typhoid | 4 | 222 |
| Enterotoxigenic *E. coli* | 1 | 1,000 |

*Table 6. Waterborne outbreaks of giardiasis compared to other waterborne outbreaks in United States, 1972-1977*

| Year | Waterborne Outbreaks of Giardiasis | Other Waterborne Outbreaks | Waterborne Giardiasis | Other Waterborne Illness |
|---|---|---|---|---|
| 1972 | 4 | 25 | 124 | 1,514 |
| 1973 | 4 | 22 | 73 | 1,701 |
| 1974 | 4 | 21 | 4,930 | 3,426 |
| 1975 | 1 | 23 | 9 | 10,870 |
| 1976 | 3 | 32 | 639 | 4,429 |
| 1977 | 4 | 30 | 1,058 | 2,802 |
| Total | 20 | 153 | 6,833 | 24,742 |

municipal systems or by persons traveling outside of populated areas (e.g., backpackers). The reporting of outbreaks involving individual water systems is much less complete than for outbreaks in municipal or semi-public water systems. The small number of documented outbreaks in backpackers is due to either the lack of investigation or incomplete investigation or reporting. When investigated, the outbreaks are often reported in an anecdotal manner. In addition, a number of single cases of giardiasis occur in which a common source cannot be investigated.

Most of the outbreaks occurred as the result of consuming untreated surface water or surface water treated only with chlorine (Table 8). The largest waterborne outbreak of giardiasis occurred in Rome, New York from November 1974 to June 1975; 350 residents had laboratory-confirmed giardiasis, and an epidemiologic study estimated that approximately 4,800 individuals were affected during the outbreak(32). Rome used a surface water source with chlorination as the only treatment. The watershed, 185 square miles of heavily wooded rolling hills, was sparsely populated, but the presence of human settlements in the watershed area suggested that the water supply could have been contaminated by untreated human waste. As previously noted, the infectivity of municipal water was confirmed by producing giardiasis in specific pathogen-free dogs fed sediment samples of raw water obtained from an inlet of a city reservoir, and a microscopic examination of the water sediments uncovered a *G. lamblia* cyst in one sample. Rome's raw water bacterial quality was generally good since the

*Table 7. Waterborne outbreaks of giardiasis in various types of water systems in United States*

| Type of System | Outbreaks | Cases |
|---|---|---|
| Municipal | 10 | 6,695 |
| Semi-public | 10 | 266 |
| Individual | 3 | 48 |
| Total | 23 | 7,009 |

*Table 8. Water system deficiencies responsible for waterborne outbreaks of giardiasis in United States*

| Deficiency | Outbreaks | Cases |
|---|---|---|
| Surface Water with Chlorination Only* | 10 | 5,307 |
| Untreated Surface Water | 6 | 186 |
| Ineffective Filtration | 2 | 1,350 |
| Untreated Ground Water | 2 | 19 |
| Ground Water with Chlorination Only | 1 | 123 |
| Contaminated Cistern | 1 | 5 |
| Unknown | 1 | 19 |
| Total | 23 | 7,009 |

* *Includes one outbreak where filtration facilities were available but used intermittently*

average raw water coliform count during the period June 1974 to June 1975 was 220/100 ml(39). Much higher than normal values (4,600 coliforms/100 ml), however, were experienced prior to the outbreak during August and September 1974, suggesting that the system may have been contaminated during this time. For water treatment, ammonia and chlorine were introduced together to produce a chloramine for a total combined chlorine residual of 0.8 mg/l. As also noted previously(21,22), chloramine is generally a less effective disinfectant, requiring longer contact times and/or higher concentrations than free chlorine. Shaw, et al (32), reported that coliform counts in the Rome distribution system were negative at 13 of 15 sampling points and 1 and 3 colonies/100 ml at the two other points. Results of routine bacteriological sampling of the distribution system showed that from November 1974 to June 1975, an average of 66 samples were collected each month; all were negative for coliforms, except 4 samples on February 25 which contained 20, 20, 30, and 40 coliforms/100 ml (personal communication, S. Syrotynski). Shaw, et al(32), also reported the total bacterial count to be quite high in the distribution system, and it is possible that this could have been responsible for the lack of high coliform counts, since high total bacterial populations have been implicated as possibly suppressing coliform growth in test media(40).

For those systems with disinfection, an attempt was made to determine if the chlorination was interrupted or the facilities were operating but providing an inadequate concentration of chlorine or contact time. Only 2 systems were identified where the chlorination facilities were defective and chlorination was interrupted prior to the outbreak. The remaining systems were apparently continuing to chlorinate with sufficient chlorine to inactivate coliform organisms but insufficient chlorine for inactivation of *Giardia* cysts.

For the untreated water system, coliform counts were available for only 2 outbreaks. In 1 that occurred in Colorado and affected 12 individuals, total coliforms of 23/100 ml were detected in the water system. The other outbreak(41) involved a group of campers on a 2-week trip in the Uinta Mountains of Utah during September 1974. Thirty-four of the 54 campers had diarrhea during and after the trip; 22 (79%) of 28 symptomatic campers' stools examined contained *G. lamblia* cysts. Epidemiological data and fecal coliform counts implicated the remote mountain stream used as a water source by the group as the vehicle of transmission. Water samples from the stream had a mean fecal coliform count of 42/100 ml. Water was not disinfected or boiled prior to consumption. The source of the infection was not identified, but several active beaver ponds, grazing sheep, and a shepherd were noted in the area. It was felt that perhaps wild or domestic animals served as an alternate host and the source of *Giardia* in this outbreak, but this could not be proven as cysts could not be identified in water or in animal droppings.

The first documented waterborne outbreak of giardiasis involving a filtered water supply occurred in Camas, Washington(33, 34), in the spring of 1976 and affected 600 individuals of a population of 6,000. Camas used 2 water sources, mountain streams and deep wells. *Giardia* cysts were isolated from the raw water entering the water treatment plant and from treated water in 2 distribution system storage reservoirs. Well water was not found to be contaminated. The watersheds for the surface water sources were well isolated, had no human habitation, and had extremely limited human activity. Several animals on the watershed were trapped and examined for *Giardia* cysts. Three positive beavers were found within foraging distance of the water intakes for Camas. Treatment for the surface water sources consisted of a mixed-media pressure filter and disinfection; no sedimentation was employed prior to filtration. Prior to the outbreak, failure of the chlorination equipment occurred, and a number of deficiencies were found in the condition and operation of the pressure filters, including ineffective chemical pretreatment. It was reported that the treated water produced by the treatment plant had met both turbidity and coliform standards prior to and during the outbreak.

A second waterborne outbreak of giardiasis involving a filtered water supply occurred in Berlin, New Hampshire(33,35) in the spring of 1977 affecting 750 of a population of 15,000. Berlin used 2 rivers for its water supply, the Ammonoosuc and the Androscoggin. *Giardia* cysts were recovered from the raw and treated water from both rivers and from 2 sites within the distribution system, the regional hospital and city hall. The Ammonoosuc River is located in the White Mountain National Forest; however, access is not restricted, and an estimated 3,000 people used the area for recreational activities during October, November, and December 1976. Water from the Ammonoosuc was chlorinated and filtered under pressure without sedimentation or flocculation. There was no pretreatment of the water with conditioning chemicals. The physical plant was 30 years old and numerous deficiencies, which could permit cysts to pass through the filters, were found in the condition and operation of the pressure filters. For the Androscoggin River, a new treatment plant with upflow clarification and rapid sand filtration was put into service just prior to the outbreak. Difficulty in creating a proper weight floc and operating the upflow clarifier were reported. An engineering evaluation revealed faulty construction of a common wall separating filtered and unfiltered water, which allowed unfiltered water to bypass the modern, conventional treatment plant. It was felt that this was the primary reason for cyst passage into the distribution system served by the modern treatment plant. Routine bacterial samples collected in the distribution system prior to the outbreak showed that the coliform standards had not been exceeded and that a free chlorine residual was not maintained in the distribution system even though free chlorine residuals of 0.3 and 0.7 mg/l were found in finished water at the treatment plants.

There were two small outbreaks involving untreated ground water systems. In one, human sewage contamination of the well was documented. The second outbreak involved a well that was adjacent to a stream. Because there was a distinct joint in the concrete casing just below the river level, the water in the well was felt to originate primarily from the stream. Algae were identified in the well water and chemical analysis of the well water and river water was quite similar. Fecal coliforms of 11 to 14/100 ml and total coliforms of 14 to 110/100 ml were found in the well water. Turbidity of the well water was 1.4-2.4 NTU. A beaver dam was located one-half mile upstream from the well. The outbreak involving a ground water system with chlorination as the only treatment was the Aspen outbreak. This outbreak and the one involving the contaminated cistern in Tennessee have already been discussed.

Waterborne outbreaks of giardiasis seem to involve 2 distinct groups of people, either visitors or campers or the usual residents of the area (Table 9).

*Table 9. Seasonal distribution of waterborne outbreaks of giardiasis in United States*

| | | Population Affected | |
|---|---|---|---|
| Month | Outbreaks | Visitors or Campers | Usual Residents |
| January | 0 | — | — |
| February | 1 | — | 1 |
| March | 3 | 2 | 1 |
| April | 1 | — | 1 |
| May | 1 | 1 | — |
| June | 4 | 3 | 1 |
| July | 3 | 1 | 2 |
| August | 2 | 2 | — |
| September | 3 | 3 | — |
| October | 0 | — | — |
| November | 2 | — | 2 |
| December | 3 | 1 | 2 |
| Total | 23 | 13 | 10 |

There appears to be a seasonal trend of outbreaks in visitors during the summer months. This implies there is either increased contamination of these water supplies during this period, or if it is assumed that the supplies are always contaminated, use by greater numbers of susceptible individuals during this period. Outbreaks involving usual residents seem to occur during any season, but most have occurred in the late fall through early spring.

There have been at least 2 major outbreaks of giardiasis in the United States where failure to isolate *G. lamblia* from the suspected water source has strongly influenced the investigators to reject drinking water as the possible vehicle of infection. One outbreak occurred in Portland, Oregon, from October 1954 to March 1955 and affected an estimated 50,000 persons(42,43). This outbreak was reported only in the correspondence section of one journal and briefly mentioned in a review article on epidemic giardiasis. A full report of the outbreak was reportedly rejected for publication by a journal on the basis of insufficient proof of the etiology of the illness and failure to isolate an organism from the suspected water supply. In retrospect, it appears that this outbreak of gastroenteritis was probably of mixed etiology with *Giardia* implicated in those cases with fairly distinctive symptoms. No enteric pathogens or viruses were isolated from clinical specimens nor were any protozoa found in unusual numbers except *Giardia*. The source of infection and mode of transmission were never satisfactorily determined although drinking water was apparently suspected. The water supply is a surface water source with chlorination as the only treatment.

The second outbreak occurred in Boulder, Colorado, from June to August 1972 affecting a population of at least 297 individuals who were positive for *G. lamblia* and had symptoms compatible with giardiasis(44). The epidemiologic information, with one major exception, did implicate a widespread common source, the exception being an age-specific attack rate heavily weighted toward young adults. A review of the records revealed no positive coliform samples, and based on a limited sampling of small volumes of water, no *Giardia* cysts were found in the water supply. Water plant operation records did indicate that because of high demand on 4 separate occasions in May and June, surface water from one source bypassed the filtration system and was treated with chlorine only for a total of 14 days. In bypassing the filtration system, the water was processed through microstrainers which cannot remove particles the size of *Giardia* cysts. Even with this information about the water treatment process, the investigators concluded that there was no evidence except a coincidence of dates to suggest that the distribution of unfiltered water was implicated as the source of infection. Unpublished data (personal communication, J. W. Hoffbuhr) also noted that the water utility was experimenting with polyelectrolyte addition prior to filtration and stopped using alum during a period before the outbreak. Because the finished water quality deteriorated during this period, alum feed was resumed. Further investigation of areas served by the unfiltered water or specific dates of change in alum feed might have provided answers to some questions raised during the investigation.

## SUMMARY

The data from waterborne outbreaks of giardiasis in the United States indicate that simple disinfection as the only treatment for surface water sources is ineffective in preventing waterborne transmission of this infection and that to protect against transmission, all surface water should receive pretreatment, preferably with sedimentation, and filtration in addition to chlorination. In Rome, the absence of filtration and treatment of surface water by simple chlorination only, left the residents unprotected against waterborne giardiasis. In Camas and Berlin, outbreaks occurred even though the water supplies were filtered. In these 2 outbreaks, deficiencies in both the operation and installation of the treatment facilities allowed the passage of cysts into the water system and subsequent illness. Lack of proper chemical pretreatment in these situations emphasizes the importance of chemical conditioning in filtration. The use of pressure filters in both of these systems illustrates the operational problems and the unreliability of pressure filter systems used for microbiological treatment of drinking water. While pressure filters are routinely used for iron and manganese removal, they are generally not recommended for microbiological treatment(45). Faulty construction of a new conventional treatment plant in Berlin allowed untreated water to bypass the rapid sand filters and illustrates the importance of good design and construction inspection. Waterborne outbreak data, engineering experience, and filtration theory indicate that well operated and properly functioning, conventional treatment plants employing coagulation/flocculation, settling, and filtration should be successful in preventing waterborne outbreaks of giardiasis. The giardiasis outbreaks that have occurred in filtered water supplies do not contradict this reasoning since numerous deficiencies have been found in the design, installation, and operation of these filtration systems. Water filtration theory indicates that organisms the size of *Giardia* cysts should be removed by conventional sand filters if effective pretreatment of the water is accomplished prior to filtration. Conventional treatment of surface water generally includes coagulation/flocculation and settling prior to filtration, or if the settling process is not used, the addition of appropriate chemicals for conditioning of the water or the filter media. New State drinking water regulations(46) adopted by Colorado in 1977 now require surface water supplies to be filtered, as well as disinfected, to remove *Giardia* cysts.

Coliform organism identification is used as an indication of fecal contamination of water supplies and is widely employed in routine surveillance programs. Negative results have usually been interpreted as providing assurance that the water is free of enteric pathogens. This interpretation must be reevaluated in light of data available from waterborne outbreaks of giardiasis. In the several outbreaks of giardiasis where disinfection was provided, *Giardia* cysts were found in the water supply in the absence of coliforms. Although adequate disinfection data are not currently available, outbreaks have shown that chlorination at conventional dosages and contact times normally employed in water treatment are inadequate for destruction of *Giardia* cysts. If *Giardia* cysts are as resistant to chlorination as cysts of *E. histolytica,* high concentrations of chlorine and long contact times would be required for cyst inactivation. Disinfection practices employed in systems using only disinfection do not provide for high concentrations of chlorine or long contact times, and in surface water systems where simple disinfection is the only treatment, it is likely that *Giardia* cysts could survive the treatment whereas coliforms would not. The coliform test in these situations could not provide assurance that an outbreak of giardiasis would be prevented.

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## Discussion

E. GELDREICH: There are 2 points I wish you would comment on which seemed to surface in your correlations with coliform occurrences or lack of them and the presence of *Giardia* outbreaks.

It appears that there is a problem of sampling frequency for small supplies, and when we investigate these small supplies in which outbreaks occurred, we may find data for only 1 to 10 samples/month. My concern is that we are not monitoring supplies with sufficient samples to recognize that there is a breakdown in treatment.

You have noted that in some outbreaks, coliforms at times range from 1 to 100/100 ml, and one wonders why there was no apparent justified response to these signals by the water treatment operator. There was something wrong, something breaking through the treatment barrier because we did find coliforms in the water. Why didn't the water plant operator or the engineers respond by doing something about it? Is it just constantly ignored because there is only a low level of coliform contamination? If so, I think we must act and sound the alarm. We cannot afford to ignore these low levels of coliform occurrences.

G. CRAUN: I agree with your comment that not enough samples are collected in the small water systems, and that more samples should probably be collected, that is, if you agree with the basic premise that by collecting samples and doing coliform tests we are going to help prevent outbreaks.

Too many times the common reaction to finding a positive is that the water sample was contaminated during collection, that either someone put their finger in the bottle or they did not collect the sample properly. A single positive sample does not seem to be taken as an indication that something needs to be done.

E. GELDREICH: Finding coliforms is not going to solve any problems in terms of outbreaks, but it should signal the water plant operator that something is wrong with the treatment and there should be a proper response. If you do find coliforms, I think there should be a sanitary engineering survey done again on the treatment system.

G. CRAUN: I agree. The reason I mentioned it was because in those systems that chlorinate only, the coliforms are disinfected but the *Giardia* cysts may not be.

E. GELDREICH: We in EPA with the Office of Drinking Water are looking at the frequency of sampling, and this certainly is an area of weakness that must be explored.

T. VERNON: I thought that was a very informative presentation, and I appreciate it. The Boulder story of 1972 is an interesting one and I think it can be well characterized as an absence of concern about transmission by water. Indeed there was a very hot late spring season in the Boulder area at that time. There was a great deal of lawn watering going on, and the records show that the slow sand filtration system was bypassed. There was a microstrainer on the system. I understand the pore size of that microstrainer would have been about 26 $\mu$m. There was no coagulation or sedimentation of that water.

I think most of the controversy in the Colorado area about the Boulder outbreak is whether indeed it was an epidemic at all. Many people believe in retrospect that with all the publicity, people just began to pick up on the endemic level of *Giardia* cysts. On the other hand, I believe most people in Boulder would say that it was an outstanding event; such an occurrence had not been experienced in preceding years, nor has it occurred in the years since that time.

One other comment which certainly leads to our discussions tomorrow on research needs is that we in Colorado have taken a step which is in a sense beyond the level of technology in that we have required complete treatment of surface waters in our regulations, interpreted to mean not only filtration but also coagulation and sedimentation. We need to know a great deal more to support our regulatory advances. This is unfortunately true in air pollution and a number of other areas in which we are instituting regulations without having the data base which we really need.

G. CRAUN: Thank you for your comments on Boulder. I was not aware that the regulations also required coagulation to precede filtration. Very good.

T. DEGAETANO: Is there any correlation between turbidity and giardiasis?

G. CRAUN: I have looked for information on turbidity in systems showing an outbreak but it generally is not available. Either the test was never performed or I simply could not obtain the information.

R. FREEMAN: All but 1 of the states that you put down on your list could be considered mountain states, whether they are east or west. Are there any outbreaks known that would be from the Plains States? Also, should we really differentiate quite clearly if we are talking about giardiasis the disease or the presence of the *Giardia* cysts in stools? I do not know if this is important. It strikes me as being important that we should differentiate between those 2 issues.

G. CRAUN: The outbreaks that are reported in the U.S. are ones in which people were symptomatic. The studies of the endemic occurence depended primarily on stool-positive individuals who may or may not have been symptomatic.

The outbreaks occurred in generally mountainous areas in all the states except Tennessee, where a contaminated cistern was involved, and there is some question as to whether or not that was actually a waterborne outbreak. I would offer an explanation of why they do not occur in the Plains, in the Midwest, in the Mid-Atlantic and the Southeast. In these areas the water sources are known to be contaminated, and appropriate treatment plants are usually built. Based on studies by CDC there is person-to-person transmission of giardiasis in daycare centers in the Southeast and places other than the mountainous areas of the country, but I think waterborne transmission is prevented because of recognition that the water supplies are contaminated and filtration is practiced. In mountainous areas where the outbreaks have occurred we have generally seen streams of fairly good quality where only chlorination was provided but not much attention was paid to maintaining the chlorinators because of a belief that the streams were not contaminated.

E. MEYER: I was not in Portland, Oregon during 1954-55, but my predecessor (L. Veazie) was, and I heard about the outbreak in some detail. In those days every medical student was asked to examine his own stool sample for parasites, and that year an extraordinarily high number of them found *Giardia*. At the same time that this epidemic came across the city, it was reported that an estimated 50,000 cases of this disease occurred, and physicians were seeing *Giardia* that they had not seen since their school days in stool samples.

My predecessor wrote the article and I am disheartened that she never published it. I have the article, and if anybody wants it or if you want to have it as an addendum to this symposium, you are welcome to it. It was at a time when there were still many people who did not believe that *Giardia* could cause disease, which could be one of the reasons that there was not much statistical information on the incidence of these cysts in people before the outbreak. In retrospect, it looks like the water supply could well have been involved. It came during the winter at a time when the rains were particularly heavy in Oregon and the water was sometimes turbid. Furthermore, I think the State Department of Public Health at the time examined these patients for viruses, for all kinds of bacteria, for other protozoa, and were not able to come up with any other possible source of diarrheal disease, but they did comment on the extraordinarily high incidence of giardiasis that they found. (*Editors Note:* L. Veazie's manuscript on the Portland, Oregon outbreak appears at the end of the Epidemiology Section on p. 174.)

# Waterborne Giardiasis

## (Summary of recent epidemiologic investigations and assessment of methodology)

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### ABSTRACT

The type and depth of epidemiologic investigations of waterborne outbreaks of giardiasis have varied considerably. The purpose of this paper is to review the strengths and weaknesses of epidemiologic investigations conducted in Rome, New York, 1975; Camas, Washington, 1976; and Berlin, New Hampshire, 1977. It is concluded that 1) an alternative case identification technique to the one presently used is badly needed, but until a new technique becomes available the presently used criteria for defining a case should be standardized so that data obtained in one outbreak are comparable to those obtained in another, 2) greater care will be required in future investigations to insure that adequate specimens are collected to rule out bacterial and viral causes of diarrhea, and 3) the SPF dog model should continue to be used to demonstrate viability and infectivity of *Giardia* cysts recovered from water until an equally reliable laboratory method is identified and made available. The long-term solution to control of waterborne giardiasis lies in improvements in and widespread use of water filtration. However, there will continue to be a need for an effective and safe method for disinfecting water on an emergency basis in communities without water filtration and in those communities where established water filters have failed.

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Although there have been numerous waterborne outbreaks of giardiasis in the United States (see Craun, these *Proceedings*) the type and depth of epidemiologic investigations performed in these outbreaks has varied considerably, and therefore the data obtained have been of varying quality. Among the more important factors contributing to the variable quality in data are: 1) the period of time elapsing between detection of a possible outbreak and initiation of an epidemiologic investigation; 2) number and training of personnel participating in the epidemiologic investigation; 3) type and availability of laboratory support services; and, 4) amount of cooperation extended by the group or community experiencing the outbreak. Early investigation of an outbreak is critical because much of the information gathered during an epidemiologic investigation is based on a respondent's memory of past events or activities. The shorter the time between the occurrence of an event and administration of an epidemiologic questionnaire, the more reliable the respondent's answers are likely to be. The number and training of personnel participating in an investigation undoubtedly have an impact. No longer can one person be expected to have all the knowledge and expertise necessary to handle all aspects of a waterborne disease investigation. Specialists are needed, and failure to seek their advice or assistance may seriously handicap an investigation. Similarly, failure to obtain adequate laboratory and community support may also undermine the investigation. In the recent investigations of waterborne giardiasis in Rome, New York; Camas, Washington; and Berlin, New Hampshire, increasing efforts were made to minimize the negative impact of these variables by enlisting the assistance and cooperation of research scientists, sanitary engineers, and health professionals at the university, local, state, and federal levels in identifying, reporting, and investigating outbreaks. While this multidisciplinary team approach has provided the scientific community with a surprising amount of new information about *Giardia* in a relatively short period of time, there is much more to be learned about the epidemiology of giardiasis.

The purpose of this paper is to review the strengths and weaknesses of epidemiologic investigations conducted in Rome, Camas, and Berlin. The objectives are three-fold: 1) to identify epidemiologic approaches that have been useful in the past so that useful data will not be omitted in future investigations; 2) to point out our mistakes so that they will not be repeated in the future; and, 3) to identify unresolved epidemiologic problems with the hope that new and innovative field and/or laboratory approaches will be found.

## ROME, CAMAS AND BERLIN OUTBREAKS - BACKGROUND

These 3 outbreaks had several features in common: 1) they all occurred in mountainous communities; 2) they all resulted from contaminated surface water, not well water; 3) chlorine was used as a water disinfectant in all 3 cities; 4) all 3 outbreaks occurred in the spring of the year (suggesting that spring thawing and subsequent overflow of stream beds may be related in some way to the outbreaks) and, 5) *Giardia* cysts were recovered from suspect water in all 3 outbreaks.

The Rome, New York, outbreak will probably be remembered for 2 reasons. First, it is the largest outbreak of giardiasis ever reported in the United States. Approximately 5,000 persons (10% of the population) were believed to have been ill. Second, it was the first investigation in which *Giardia* cysts were actually recovered from water during an outbreak(1). The Camas outbreak differed from the one in Rome in that: 1) it was the first outbreak involving a filtered water supply; 2) it was the first outbreak in which *Giardia* cysts were readily demonstrated in samples of raw and treated water; and 3) it was the first time that an animal reservoir (beavers) had been implicated as a source of water contamination(2). The outbreak in Berlin was unique among the 3 in that about half of the persons infected with *Giardia* were asymptomatic. Berlin also differed from the other 2 communities in that it had 2 surface water supplies, each with its own water treatment facility that included filtration. *Giardia* cysts were found in raw and treated water from both water supplies, and there was some epidemiologic evidence to suggest that there may have been 2 simultaneous waterborne outbreaks in the city(3).

## EPIDEMIOLOGIC DATA RELATED TO WATER

Epidemiologic data related to water are summarized in Table 1. The amount of chlorine used in routine water disinfection is believed to have virtually no effect on *Giardia* cysts. However, it is important that both total and free residual chlorine levels in drinking water be measured in waterborne outbreaks. The determination that adequate chlorination of water exists provides additional epidemiologic evidence that a waterborne epidemic of diarrheal disease is due to *Giardia*, since most bacterial and viral agents of diarrheal disease would be killed by adequate levels of chlorine. The Rome, New York, water supply was inadequately chlorinated. Free residual chlorine could not be detected at the time of the investigation. The New York State chlorination standard is 0.2 mg/l of free chlorine or 1 mg/l of chloramine throughout the distribution system. Inadequate chlorination during this outbreak indicates that possibly some of the illness within the community may have been due to bacteria or viruses. During the outbreak in Camas, several temporary breakdowns of an automatic chlorinator also resulted in undetectable levels of free residual chlorine in the water distribution system on at least 1 day(4). Adequate chlorine levels were found in the Berlin water system throughout the epidemic.

Water filtration plays a key role in preventing waterborne outbreaks of giardiasis. However, outbreaks have occurred in at least 2 communities that used some type of water filtration procedure. Types of water filters and their filtering efficiency are discussed elsewhere in these *Proceedings*. It must be

*Table 1. Waterborne epidemics of giardiasis*
*(Epidemiologic data related to water)*

| VARIABLE | 1975 ROME, N.Y. | 1976 CAMAS, WASH | 1977 BERLIN, N.H. |
|---|---|---|---|
| Type of water | Surface | Surface | Surface from 2 sources |
| Water chlorinated | Yes - but inadequate | Yes - but temporary interruptions | Yes - satisfactory at all times |
| Water filtered | No | Yes | Yes |
| Type of filtration | | Pressure filter | 1) Pressure filter<br>2) Gravity sand filter |
| *Giardia* cysts recovered from water | Yes | Yes | Yes |
| Recovery method | CDC sand filter | EPA filter | EPA filter |
| Identification method | Inoculation of SPF dogs | Microscopic exam of filter sediment | Microscopic exam of filter sediment |

emphasized that in the 2 outbreaks where water was filtered prior to distribution, defects were found either in the filters themselves or in their utilization. For water filters to be effective in removing *Giardia* cysts, they must be properly constructed, properly maintained, and properly operated. Because this is not always the case, it should never be assumed that the presence of water filtration devices rules out the possibility of waterborne giardiasis.

Epidemiologic evidence for waterborne transmission for *Giardia* has existed for many years, but definitive proof, i.e., recovesy of the organims from suspect water, managed to elude investigators until 1975. At the time of the Rome, New York, outbreak, the Center for Disease Control (CDC) was in the process of evaluating a water sampling sand filter. Although very little was known about the capability of this filter, the CDC decided to use it in Rome. The sand filter is capable of handling 95,000 liters (25,000 gal.) of water per day. Filtration of the water in Rome yielded approximately 1 liter of sediment in a 24 h period. The filter was operated for 10 consecutive days providing 10 different sediment samples. Approximately 30 coverslip preparations were made from each sample and examined in Rome by a CDC microbiologist within 12 h after collection. No *Giardia* cysts were found by direct microscopy in Rome. However, 2 of 10 samples sent to Colorado State University for inoculation into specific pathogen-free (SPF) beagles did produce asymptomatic infections in the dogs. Aliquots of the 2 samples found infective for dogs were then reexamined microscopically. After 40 coverslip preparations, the CDC lab was able to find 1 *Giardia* cyst in 1 sample; no organisms were seen in 20 coverslip preparations from the second sample. In the other two outbreaks, *Giardia* cysts were easily recovered from both raw and filtered water and identified by simple microscopic techniques. A new piece of portable filtration equipment developed and operated by the Environmental Protection Agency (EPA) investigative team is credited for this great improvement in water sampling technology (see Jakubowski, these *Proceedings*). The CDC sand filter was also used in Camas, Washington, but no *Giardia* cysts were recovered using this equipment.

An attempt was made in Camas to demonstrate the infectivity of *Giardia* cysts recovered from water, using SPF beagles. Unfortunately, specimens were mishandled enroute to Colorado and when they were received by Colorado State University obvious morphologic deterioration of the cysts had occurred. The specimens were given to the beagles anyway but failed to produce patent infections. SPF beagle studies were not performed during the Berlin outbreak due to the unavailability of dogs at that time.

The contribution made by the SPF dog model in the Rome investigation should not be overlooked in future outbreaks. Although use of SPF dogs is relatively expensive, this biologic method for recovering *Giardia* yields important information not available by other techniques. Direct microscopic examination of filter sediment provides information about the presence or absence of *Giardia* cysts in the water, but it provides no information about the animal origin of the cyst, the viability of the cyst, or its potential infectivity for humans. Using in vitro excystment procedures (see Bingham, these *Proceedings*), some assessment of cyst viability is currently possible, but the relationship between excystment and infectivity of *Giardia* cysts has not yet been established. Thus, the SPF dog model is the only method that demonstrates both viability of *Giardia* cysts and their infectivity. From the studies of Davies and Hibler (see Davies, these *Proceedings*), there is also suggestive evidence that cysts found infective for SPF dogs are potentially infective for humans.

## SOURCE OF WATER CONTAMINATION

Once a *Giardia* cyst has been identified in water, the next logical question is, "How did it get there?" Data on human and animal sources of water contamination are summarized in Table 2. In Rome, Camas, and Berlin, there was no evidence of sewage contamination of drinking water. The most likely source of water contamination in Rome was never determined. Water Department personnel were confident that there were no humans living in the watershed area. However, aerial reconnaissance revealed several cabins along the shore of the lake serving as the main storage reservoir. It was recommended that the method of sewage disposal used by cabin occupants be evaluated and a special search be made for evidence of sewage discharged directly into the lake. To my knowledge this was never done. Similarly, a stool survey to determine the prevalance of *Giardia* in humans and animals in the watershed was proposed but never performed.

In Camas, drinking water was supplied by 2 small streams. The watershed area was so steep and heavily forested that it could only be entered by walking up the middle of the stream bed. The stream beds were explored to their origins and the watershed area was also surveyed from the air. We

*Table 2. Source of* Giardia *cyst contamination in waterborne epidemics of giardiasis*

| SOURCE OF WATER CONTAMINATION | 1975 ROME, N.Y. | 1976 CAMAS, WASH. | 1977 BERLIN, N.H. |
|---|---|---|---|
| Sewage cross-connection | No | No | No |
| Human | Undetermined but possible | Unlikely | Likely |
| Animal | Undetermined but possible | Likely | Possible |
| Beaver | | 3/7* | 1/5 |
| Otter | | | 0/5 |
| Mink | | | 0/4 |
| Porcupine | | 0/1 | 0/1 |
| Coyote | | 0/2 | |
| Opposum | | 0/1 | |
| Nutria | | 0/1 | |

* *No. positive/No. examined*

found no evidence of human trespassers and no houses in the watershed area. Since human contamination seemed unlikely, the possibility of an animal reservoir was considered. Three of 7 beavers captured had *Giardia* in their stool. This was the first time that *Giardia* had ever been recovered from a beaver. *Giardia* cysts recovered from the beavers and those recovered from the city water supply appeared morphologically identical to *Giardia lamblia* obtained from infected humans. In addition, beaver *Giardia* were found to be infective and cause disease in SPF beagle puppies at Colorado State University.

The fact that infected beaver were found below, instead of above, the water intakes weakens the epidemiologic hypothesis that they were the source of infection (Fig. 1). However, beaver are known to migrate in the spring, and we found several caches of freshly cut limbs above the water intakes to attest to the recent presence of beavers in those locations. It is hypothesized that beaver may have become infected through exposure to water contaminated with human feces further down stream and that during their spring migration the beavers contaminated the streams above the water intakes, thus, precipitating the outbreak.

In Berlin there was ample opportunity for human fecal contamination of raw water sources. Although 1 of 5 beaver captured in the water area was infected with *Giardia*, there was no way to determine whether this animal was an unlucky victim of water contaminated with *Giardia* of human origin or whether beaver served as a major contributing source of *Giardia* in the water.

## CLINICAL-EPIDEMIOLOGIC PROBLEMS

Two particularly difficult clinical-epidemiologic problems have been encountered in investigations of suspected waterborne giardiasis. The first is development of an accurate case definition, i.e., the epidemiologist must have some method for deciding who does or does not have giardiasis. The second problem is to determine whether *Giardia* is the real cause of illness or merely a commensal organism. In a clinical setting it is not difficult to determine if a patient is infected with *Giardia*. A physician simply requests multiple stool exams or, in some circumstances, obtains a duodenal aspirate or biopsy. However, in an epidemiologic study, it is not possible to obtain 1 stool specimen, let alone 2 or 3, from each of 400-1000 survey respondents. Since there is currently no suitable serologic test for giardiasis, epidemiologists have had to rely heavily on a respondent's description of his or her illness as a diagnostic method. Unfortunately, the symptom complex in giardiasis is not pathognomonic for the disease. Several bacterial and viral agents produce similar illnesses.

The chronic nature of diarrhea in giardiasis is the one clinical feature that has been used to differentiate giardiasis from bacterial and viral enteritis. The minimum duration of diarrhea accepted by investigators as being indicative of giardiasis has varied from one outbreak to another (Table 3). In Rome, a clinical case of giardiasis was defined as any person who had

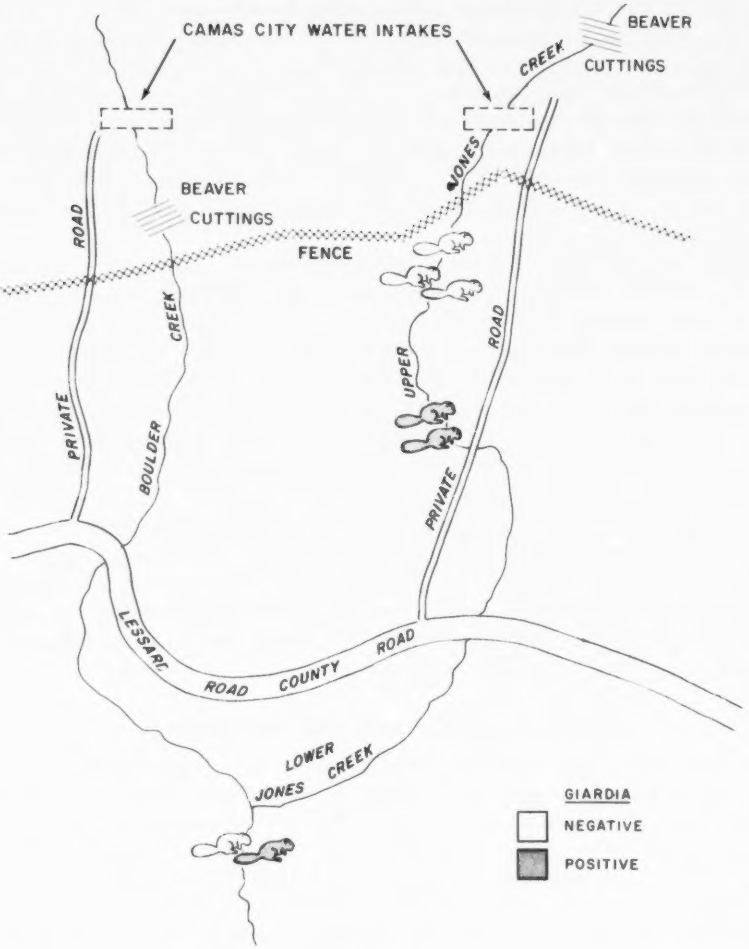
CAMAS CITY WATER INTAKES
BEAVER CUTTINGS
CREEK
BEAVER CUTTINGS
JONES
FENCE
ROAD
PRIVATE
BOULDER CREEK
UPPER
ROAD
PRIVATE
LESSARD
ROAD COUNTY ROAD
LOWER JONES CREEK
GIARDIA
NEGATIVE
POSITIVE

FIG. 1. *City of Camas Watershed, May 1976.*

diarrhea for 5 days, or more. In retrospect, this case definition may be too broad. Evidence is increasing that certain bacterial infections (*Campylobacter*) and viruses (rotavirus) may cause diarrhea of long duration. In an English study of *Campylobacter*-associated enteritis in over 1000 persons, 8% had persistent or recurring diarrhea lasting 2 or more weeks(5). While the frequency of acute abdominal pain and blood in stools can be useful in distinguishing *Campylobacter* enteritis from giardiasis, these clinical features are not consistently present. In the above study less than 15% of patients with *Campylobacter* infection reported these symptoms or signs. Thus, many *Campylobacter* infections could be mistaken for giardiasis. Similarly, while rotavirus is commonest in young children and usually causes diarrhea for less than 10 days, it does occur in persons of all ages and may produce diarrhea lasting 5 days to 3 weeks (usually 8 days)(6). Since no specific search was made for *Campylobacter* or rotavirus in Rome and chlorination of Rome water was inadequate, the possibility exists that 1 of these organisms may have contributed to the size of the outbreak. Tightening the clinical case definition for giardiasis to a minimum of 10 days of diarrhea would not have completely excluded bacterial and viral cases of enteritis from consideration; however it would have reduced the probability of their being included in numbers large enough to have had a significant influence on statistical analysis.

*Table 3. Clinical-epidemiologic findings in recent waterborne outbreaks of giardiasis*

| | 1975<br>ROME, N.Y.<br>(Pop. 50,000) | 1976<br>CAMAS, WASH<br>(Pop. 6,000) | 1977<br>BERLIN, N.H.<br>(Pop. 15,000) |
|---|---|---|---|
| Clinical case definition: Duration of diarrhea in days | ≥5 | ≥10 | ≥7 |
| Clinical attack rate (No. surveyed) | 10.6% (1421) | 3.8% (503) | 5% (676) |
| Stool positivity rates (No. surveyed) | 3.4% (645) | 11% (18) | 46% (74) |
| Stool positive but asymptomatic (No. surveyed) | N.D.* | 0% (9) | 45% (51) |

* *Not done*

It should be kept in mind that these observations and criticisms are being made through the power of hindsight. At the time of the Rome outbreak our concept of the public health significance and clinical spectrum of *Campylobacter* and rotavirus was quite different from that of today. Based on other epidemiologic data gathered during the outbreak, I feel confident that *Giardia* was the principal etiological agent involved, but there is no assurance that *Giardia* was the only cause of diarrheal illness lasting 5 or more days.

In Camas, we attempted to increase the specificity of the case definition for giardiasis by accepting as cases only those persons who had diarrhea for 10 or more days. In Berlin, data were analyzed using a clinical case definition of diarrhea lasting 7 or more days; few people in Berlin had illnesses lasting 10 or more days. The clinical attack rates based on the above clinical case definitions are shown in Table 3. To some extent the attack rates are inversely proportional to the number of days of diarrhea used to define a clinical case of giardiasis.

One of the strong points of the Rome investigation was the large number of stool specimens that were obtained from survey respondents (Table 3). Of 1421 persons surveyed, 645 submitted a stool specimen and 3.4% were positive for *Giardia*. Unfortunately, the proportion of asymptomatic persons surveyed who were positive for *Giardia* was not tabulated. This can be a very useful figure, especially if calculated during the field investigation. A high rate of stool positivity in asymptomatic persons is suggestive of an endemic *Giardia* problem rather than an epidemic one. It alerts the epidemiologist, early in the investigation, to the possibility that *Giardia* may not be the cause of illness, thus making it possible to arrange special studies to resolve the issue. We attempted to do this in the Camas and Berlin investigations.

We requested stool specimens from 20 persons in Camas and received them from 18. Nine of the 18 persons had a history of diarrhea of at least 2 days duration and 9 were well. Of the 18 persons, 2 (11%) had *Giardia* in their stools; both persons had a history of diarrhea fitting the clinical-case definition, i.e., diarrhea lasting 10 or more days. *Giardia* was not found in stools of asymptomatic persons.

The stool positivity rates in Berlin were quite different from those obtained in the previous outbreaks. Eleven percent (74/676) of the survey respondents submitted a stool specimen for examination, and *Giardia* was found in 46% of these stools. Sixty-nine percent (51/74) of the persons submitting a stool specimen were asymptomatic and yet 45% also had a *Giardia* infection. By repeating the stool survey 9 months later, we were able to demonstrate that the high rate of stool positivity in asymptomatic persons was probably not a reflection of an endemic problem. In the second survey of 54 randomly selected asymptomatic individuals, only 2 (3.4%) were infected. The data did sensitize us to the fact that a careful search for other possible etiologic agents would be essential to the investigation. The methods used to rule out other enteric pathogens are discussed in the next section.

The Berlin epidemic also provided us with a unique opportunity to evaluate the role of person-to-person transmission of *Giardia* within families. Eight stool-positive persons worked in Berlin but resided in nearby towns. We examined the stools of all 23 of their household contacts and found none positive for *Giardia*. This indicates that person-to-person transmission of *Giardia* during a waterborne outbreak does not significantly contribute to further dissemination of the parasite.

## RULING OUT OTHER CAUSES OF DIARRHEA

Recent clinical and epidemiologic data support the concept that *Giardia* is a pathogenic parasite of humans. However, it must also be acknowledged that *Giardia* infection does not always result in clinical illness. For this reason, finding *Giardia* in persons with diarrhea during an epidemic has not been accepted as *prima facie* evidence that *Giardia* is the cause of illness. In order to assess the relationship between *Giardia* infection and clinical illness in epidemics, a number of epidemiologic approaches have been tried. One of the most common approaches has been a retrospective analysis of laboratory reports. An increase in the frequency of diagnosis of a particular enteric pathogen in a community coincident with an increase in diarrheal disease suggests that the organism may be related to the outbreak. This is a very useful and practical technique, but it does have one drawback — most local and many state laboratories do not include the more difficult to isolate bacteria (*Campylobacter*, enteropathogenic *E. coli*, *Yersinia*) and viral agents (rotavirus, Norwalk, etc.) in their routine examinations of stool. Therefore, it has been argued that an increased diagnosis of *Giardia* by a laboratory during a waterborne outbreak of diarrheal illness may only reflect fecal contamination of the water supply and that an undiagnosed bacterial or viral agent is the actual cause of illness. The probability of this occuring is highest where chlorination of water is not adequate to kill bacterial and viral contaminants.

Another method that has been used to rule out other pathogens is to look for them in ill patients with stool-confirmed giardiasis. If *Giardia* is not the cause of illness, then one would hope to demonstrate the real cause. This is a valid technique provided 1) the laboratory used is capable of culturing and identifying the more difficult-to-isolate organisms, and 2) adequate specimens from a control group (persons without diarrhea and without *Giardia* in their stools) are obtained at the same time in case another organism is detected in stool-confirmed cases of giardiasis. The second provision has not been closely adhered to. We have been fortunate that no other pathogens have ever been recovered in significant numbers from stool-confirmed cases of giardiasis.

The third and probably best technique for determining the true cause of diarrhea in an outbreak is to randomly select 10 to 20 acutely ill persons and 10 to 20 persons without illness for a complete parasitic, bacterial, and viral stool examination. The specimens must be obtained at approximately the same time (within 7 to 10 days). For this approach to succeed, it is imperative that persons planning and/or executing specimen collection know the type of specimen(s) to be collected (stool, rectal swab, serum) and become thoroughly familiar with factors that make specimens unsuitable for laboratory examination such as recent use of antidiarrheals or antibiotics, recent barium studies, elapsed time between onset of illness and specimen collection and between collection and preservation of specimens, type of preservative (fixatives, transport culture media, freezing, etc.), and appropriate packaging and transport time for laboratory samples.

In all 3 outbreaks discussed here, retrospective analysis of laboratory reports indicated an increase in the number of stools positive for *Giardia* concommitant with an increase in diarrhea in the community. The most intensive search for other enteric pathogens in stool confirmed cases of giardiasis was conducted during the Camas investigation. All 128 stool-confirmed cases of giardiasis identified by local laboratories in that outbreak were cultured for *Salmonella* and *Shigella*. *Salmonella* was isolated from only 1 person. Seventy-five of the 128 confirmed cases of giardiasis had viral cultures; all were negative. However, local laboratory examinations did not include a search for toxigenic and invasive *Escherichia coli, Yersinia, Campylobacter,* or rotavirus. These studies were performed at CDC on stool specimens obtained from 9 ill persons and 9 well persons selected at random from the community. *Giardia* was found in 2 of the 9 ill persons; no other enteric pathogens were identified in either group.

In Berlin 20 fresh diarrheal stool specimens arriving at the local hospital laboratory during the epidemic period were cultured for *Salmonella* and *Shigella* and found to be negative; *Giardia* was identified in 11 of these stool specimens. In addition, CDC examined a fresh stool specimen from each of 12 randomly selected Berlin residents with a recent history of diarrheal illness; 5 of these specimens were collected within 1 week of illness. All specimens were negative for *Salmonella, Shigella,* toxigenic and invasive *E. coli,* pathogenic vibrios, *Yersinia enterocolitica,* and *Campylobacter jejuni.* Virologic studies included examination of fresh frozen diarrheal specimens obtained within 1 day of onset of illness in 6 Berlin patients ranging in age from 11 to 70 years. Rotavirus-like particles were identified in an 11-year-old boy who was also infected with *G. lamblia.*

## FUTURE NEEDS AND CONSIDERATIONS

The clinical case definition of giardiasis used in epidemiologic investigations and based on duration of diarrhea leaves much to be desired. Alternative case identification techniques suitable for epidemiologic investigations of giardiasis such as a serologic test or a method for detecting *Giardia* antigen on rectal swabs are needed. During future epidemics of giardiasis, evaluations should be made of newly developed diagnostic tests. However, until new case identification techniques become available, an attempt should be made to standardize the clinical case definition used for epidemiologic purposes so that data obtained in one outbreak are comparable with data obtained in another. Diarrhea lasting for 7 or more days is suggested as the minimum duration of diarrhea that should be considered as indicative of a clinical case of giardiasis. Of course, the patient's illness must be otherwise compatible with giardiasis as well. When there is evidence of inadequate chlorination of water or in the absence of good laboratory support to rule out other enteric pathogens, the minimum duration of diarrhea used in the case definition should be extended to 10 or more days. It should be recognized that some *Giardia* infections will result in diarrhea of a much shorter duration and will be excluded from study by these case definitions.

The clinical differences in diarrheal disease caused by certain protozoan, bacterial, and viral agents appears to be decreasing as the amount of new clinical and epidemiologic information about enteric pathogens increases. Therefore, greater care will be required in future investigations of waterborne outbreaks of diarrheal disease to insure that adequate specimens are collected for parasitic, bacterial, and viral studies, regardless of the suspected etiology of the outbreak.

We should continue to use the SPF dog model to demonstrate the viability and infectivity of *Giardia* cysts recovered from water in future outbreaks, at least until equally reliable laboratory methods, such as excystation of *Giardia* cysts in vitro or vital staining, can be perfected and their correlation with results obtained with SPF dogs is established. We should not lose sight of the fact that *Giardia* cysts found in water may come from a variety of different animals and that not all of these cysts are infectious for humans (see Davies, these *Proceedings*). At the present time, the only method for assessing whether *Giardia* cysts recovered from water are potentially infectious for humans is to demonstrate that they are infectious for dogs.

More research is needed to elucidate those factors that contribute to the development of clinical giardiasis. Parasite factors that deserve further study include dose, strain differences relating to geographic regions, and origin (human or animal). Host immunity undoubtedly influences the risk of developing illness, but the exact mechanisms have not been determined. Outbreaks of giardiasis provide excellent opportunities to study specific immune factors that may play a role. Development of a simple technique for evaluating host immunity for application in epidemiologic investigations deserves further consideration.

The long term solution to control of waterborne giardiasis will undoubtedly involve improvements in widespread use of water filtration. However, effective management of emergency problems encountered in waterborne outbreaks also will be needed, particularly in communities where water filtration has never been implemented as well as in communities where existing filtration equipment has failed and cannot be repaired promptly. Under these circumstances, safe and effective methods for destroying *Giardia* cysts in drinking water are certainly needed. Techniques for making a field evaluation of the efficacy of existing water filtration equipment would also be helpful. Emergency methods for handling animal reservoirs implicated as a source of waterborne outbreaks deserve further consideration. Killing these animals is neither a desirable nor an effective emergency procedure. Perhaps an effective way to administer antigiardial medication to target animals in food (e.g., apple pulp for beaver) could be explored.

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## Discussion

R. RENDTORFF: I would like to thank you for a fine presentation. My only objection was that it did not come before the others so we could evaluate those better. The large number of cases reported in Rome, as you pointed out, is extremely important to epidemiology.

One thing that intrigued me is that you attempted to determine if *Giardia* were spread by the individuals who worked in Berlin, N.H. among those within their households that lived out of the Berlin area. I am wondering if the shedder was a food handler, and if your sampling from household members was at the time when shedding occurred. In our volunteer experiments some of these infections are extremely transitory and last but a few days.

D. JURANEK: We did get into the investigation a little bit earlier than some of the other outbreaks, so there were ongoing cases at the time the investigation was conducted. As I recall, these 8 individuals that worked in Berlin but lived outside the community were all men. I am making a supposition that they may not have been food handlers in the household, although they were excreting *Giardia* cysts shortly before we examined household members. We only did 1 stool exam on household members, however, and there is some question about the reliability of just doing a single stool exam.

M. SCHULTZ: Also, in the studies that we did of the tourists that went to Leningrad and came back with giardiasis, there was virtually no clinical evidence of secondary spread from them to their family members.

T. VERNON: Recently there was a study conducted on next door neighbor families who were matched to families in which there were cases, and the prevalence rate in the families next door was the same as the prevalence rate of the family members of the known cases.

D. PRICE: I am working with a migrant community, and I would like to know how you would classify endemic and epidemic people in a migrant situation in which you had fluctuating rises and falls in giardiasis.

D. JURANEK: That is going to be a difficult problem. I would suspect you would have to follow these same people throughout their migratory pathway and see what happens, especially if you have treated them once and then they go into another community and experience the same prevalence of infection. You have to establish whether they are all getting giardiasis from an environmental source or whether there is a certain amount of person-to-person transmission within the families. In a migrant labor situation it would not surprise me if there was some person-to-person transmission within the household simply because they usually live under poor sanitation and hygienic conditions.

D. PRICE: It is my understanding that the chlorine content has been reduced in the last few years in water supplies. Many of the health department examinations are not for free chlorine but are for total chlorine. Does this give us an indication of what is actually going on in the water supply, particularly in areas where you have a high organic content in the water?

D. JURANEK: That is my impression, but I want to defer to the EPA experts; they will have to address that.

G. HEALY: Your point about examining asymptomatic individuals in a population with an outbreak is very important. Would you consider, as an epidemiologist, that it is worthwhile to conduct a study for the detection of *Giardia* cyst in water supplies, and do you know of any situations today where a selected city is now being evaluated? Do you know if EPA has a filter in use anywhere to determine the presence of *Giardia* cysts?

D. JURANEK: I believe there is one such study being performed under EPA funding in the State of Washington.

# Water Supply Problems Associated With a Waterborne Outbreak of Giardiasis

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## ABSTRACT

A waterborne outbreak of giardiasis occurred in the spring of 1977, in Berlin, New Hampshire. Investigation of the water system identified a source of contamination, recovered *Giardia* cysts in samples of raw and treated water, and determined routes by which cysts passed through the treatment plants. Corrective measures were implemented to abate the outbreak. Other problems related to the operation of water systems and the transmission of *Giardia* cysts are discussed.

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A waterborne outbreak of giardiasis occurred in the spring of 1977 in Berlin, New Hampshire. Results of an epidemiological study indicated that nearly 7,000 of the city's 15,000 residents were infected with *Giardia lamblia(1)*. Large volume samples of raw and treated water collected from the water system were positive for *Giardia* cysts and routes by which the cysts passed through the two plants were found. A survey of one of the watersheds used as a source of supply revealed a beaver lodge in the raw water storage reservoir. One of 4 beavers inhabiting the lodge was infected with *Giardia(2)*.

This paper will deal with some of the problems encountered in the investigation of the Berlin Water System and other concerns for water supplies which are challenged by *Giardia* cysts.

## DESCRIPTION OF THE BERLIN WATER SYSTEM

Berlin is supplied water from two sources; the Upper Ammonoosuc River and the Androscoggin River. The Upper Ammonoosuc River, located west of Berlin, is impounded by Godfrey Dam which forms a water supply reservoir of 99.4 x $10^3 m^3$ (26 MG) capacity. The heavily wooded watershed is located within the confines of the White Mountain National Forest and under control of the National Forest Service. There are no point-source discharges upstream of the reservoir; however, hunting, fishing, and other forms of recreation are permitted. Water is transported from the reservoir through a bottom intake to a treatment plant located on the western fringe of the city as shown in Figure 1.

The Ammonoosuc Treatment Plant consists of eight pressure filters of cylindrical shape that are 2.4m (8 ft) in diameter and 5.5m (18 ft) in length. The filters contain graded anthrafilt media and are operated in parallel fashion. Chemical conditioning of water prior to filtration is not practiced.

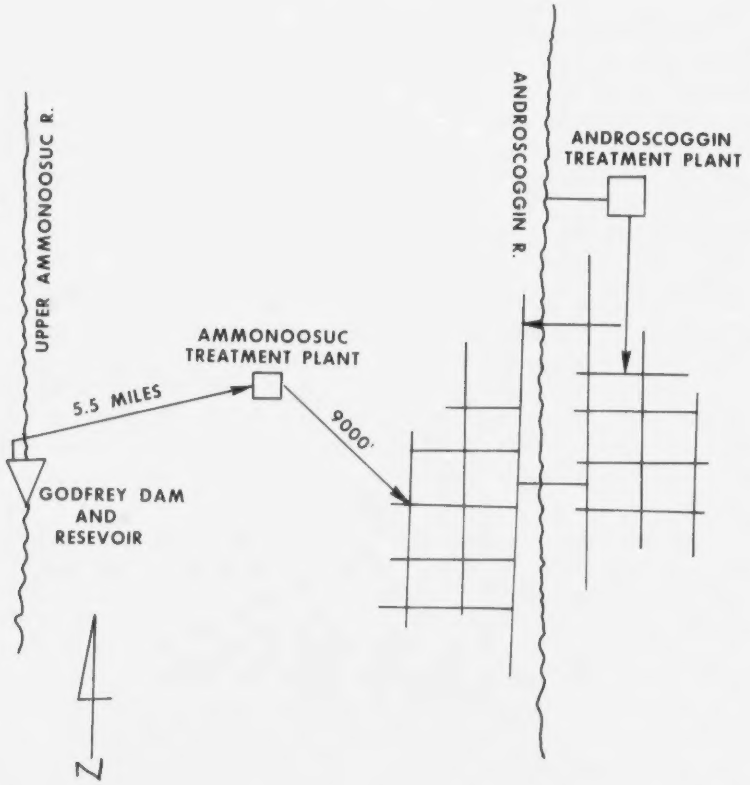
UPPER AMMONOOSUC R.
ANDROSCOGGIN R.
ANDROSCOGGIN TREATMENT PLANT
AMMONOOSUC TREATMENT PLANT
5.5 MILES
9000'
GODFREY DAM AND RESEVOIR
N

FIG. 1. *Simple schematic diagram of Berlin, N.H. water system.*

Filter effluent is chlorinated and the treated water is introduced into the distribution system that mainly serves the western portion of the city.

The Androscoggin River, which is the second source of supply, flows through the city and has a drainage area of approximately 3367 km$^2$ (1,300 sq mi). Average flow at Berlin is 67m$^3$/sec (2,400 cfs). The drainage area is mostly timberland and there are no significant point-source waste discharges upstream of the treatment plant.

Water is pumped from the river to a recently completed treatment plant. Conventional treatment is provided including chemical coagulation, clarification, rapid sand filtration and chlorination. Treated water is distributed mainly to the eastern portion of the city; however, there are several river crossings and a zone of mixed water occurs in the system adjacent to the Androscoggin River.

The city had not experienced any unusual water quality problems prior to and during the outbreak as measured by conventional parameters for raw or finished water. Coliform organisms were not detected in finished water based on 5 weekly samples and historically were extremely low for both raw water sources usually occurring in the range of less than 100 colonies/100 ml. Turbidity values for raw water were normally less than 5 Nephelometric Turbidity Units (NTU) with fluctuation occurring during periods of runoff. The values were acceptable for finished water when compared with applicable standards at that time. There was a slight color problem in finished water from the Ammonoosuc source presumably resulting from decaying vegetation.

## WATER SUPPLY PROBLEMS

*Giardia* cysts were detected in large volume samples of raw and finished water from the Ammonoosuc and Androscoggin treatment plants and in samples of finished water from several locations in the distribution system. The results presented a number of problems including: source of the contamination, the reason for passage through the treatment plants, abatement procedures that could be utilized to prevent further illness, and other concerns that deserve attention.

### Source of Contamination

It was reasonable to conduct a sanitary survey in the Ammonoosuc watershed to determine the source(s) of contamination because of its size but the prospect of inspecting the entire Androscoggin drainage area (1,300 sq mi) was not seriously considered. Inspection of the perimeter of Godfrey Reservoir showed a beaver lodge about 610m (2000 feet) upstream from the Ammonoosuc plant intake. Four beavers were eventually trapped with permission of the State Game Commission and autopsied at a local hospital.

Samples of material from the gastrointestinal tract of the animals were initially examined and the findings were negative. The pathologist then prepared slides from material extracted from the duodenal mucosa and positively identified *Giardia* trophozoites in one of the beavers.

Trapping and sacrifice of wild animals during the outbreak was unpopular. Resentment existed in the public and governmental agencies not aware of the implication of beaver in a previous outbreak of giardiasis in Camas, Washington(3). There are alternatives to sacrifice of animals such as collection of fecal specimens from their habitat, or live trapping the animals for study under captivity. If the animals are actively shedding cysts, the results are soon available; however, they may be infected and not shedding cysts as was the case with the Godfrey Reservoir beaver. There is also the question of resources to support continued study of an animal in captivity where its habits and shedding conditions may change, and the length of time that one can wait for results. Collection of fecal samples from the vicinity of the beaver lodge was not attempted. The water in this area was 3 to 4.6m (10 to 15 ft) deep with a temperature of about 2 to 3°C and there were no volunteers available to obtain a fecal sample.

It would have been desirable to conduct profile sampling of the Androscoggin River to locate sources of *Giardia* but there was not sufficient laboratory support for sample analyses. A limited sanitary survey was made and over 40 cease and desist orders were issued to homes and institutions with individual sewage disposal violations.

Based on the limited information available it was concluded that the source of cyst contamination in the Ammonoosuc watershed was beaver, possibly infected by human carrier. The source of cyst contamination in the Androscoggin River was unknown.

## Cyst Passage Through Treatment

*Giardia* cysts were detected in raw and finished water at both treatment plants during the outbreak using sampling methodology developed by EPA(4). Treatment processes and facilities were evaluated to determine how cysts passed through the plants.

The Ammonoosuc Plant relied solely on filtration for particle removal while chemical coagulation and clarification were provided prior to filtration at the Androscoggin Plant. The effectiveness of either form of treatment for *Giardia* cyst removal had never been investigated. Cysts of *E. histolytica* which are similar in size to *Giardia* cysts have been studied in pilot plant experimentation(5). The researchers found that *E. histolytica* cysts were removed by conventional treatment similar to that provided at the Androscoggin Plant, and that they were not removed by simple filtration. These results are somewhat reinforced by experimentation conducted by the Center for Disease Control during studies of sampling methodology for *Giardia* cysts in drinking water. The method consisted of passing a large volume of water through a swimming pool filter in which the normally used diatomaceous earth medium was replaced with 0.45 m (18 in) of 0.45 mm sand. In a laboratory experiment, *Giardia* cysts at a concentration of 20/3.8 l (1 gal) were dosed onto the filter and after passing 34,200 l (9,000 gal), no cysts were detected microscopically in the concentrated preparations of filter backwash(6).

When filtration is used as the only treatment process, removal of applied material depends on the mechanism of straining. While forces of sedimentation do occur in channels and voids in the filter bed, the predominant mode of removal is physical straining or trapping of particulate matter. The trapping capability of a filter bed can be visualized by considering the size of the voids. In a filter containing sand with a diameter of 0.5 mm, the pore openings or voids will range in size from 0.1 to 0.2 mm. With coarse angular filter media such as carbon-based material, the pore openings are larger, ranging from 0.3 to 0.6 mm. Particles that are easily trapped are larger than the pore opening while smaller particles may pass through. *Giardia* cysts measuring 10 to 14 $\mu$m can be easily transported through pore spaces that are 10 to 40 times their size. For filtration to be effective in removing small particles, it should be preceded in the treatment train by chemical conditioning or coagulation/flocculation. This enables the formation of larger particles and permits filtration to reduce the applied load of turbidity and microbial populations through mechanisms of straining, sedimentation and flocculation in the voids, and adsorption.

The passage of cysts through the Ammonoosuc Plant was accommodated by the condition of the filter beds. Pressure filters are enclosed vessels and inspections of the filter beds are infrequent because access is difficult. Inspection of the filters during the outbreak showed that three had severely disrupted bed surfaces characterized by mounds, pyramids, and shrinkage resulting in separation of the beds from vessel walls. The filters were plagued with agglomeration of mud masses within the beds and extended backwashing sequences were not successful in cleaning the media. One filter had a large conical depression covering approximately one-third of the bed area which resulted from a break in the main header of the air wash system. During backwash, air that was normally distributed uniformly across the bed cross-section for scouring action, was discharged full-force from the header break. The disrupted bed conditions changed the profile of filter surfaces and filtration occurred through only a few inches of media instead of 61 cm (24 in) of a normal filter bed.

Passage of cysts through the Androscoggin Plant was not expected because the type of treatment was thought to be effective for removal. The passage of cysts was initially thought to be related to inefficient control of coagulation and solids removal prior to filtration. This possibility was investigated and resulted in a recommendation by the process designers that other chemicals be used. However, further study of plant operation detected escape of bubbles through the joints in the slab of the backwash channels during the filter washing sequence (Figure 2). The washing sequence included the introduction of air into the filter bottom to permit scouring and cleansing of the filter media. The escape of air through the joint indicated that water applied to the filters could be by-passing treatment and contaminating filtered water. It also indicated that during the backwash sequence, water laden with concentrated filterable material including microorganisms would pass through the joint and contaminate filtered water. Leakage through the joint was confirmed by static hydraulic test of the backwash compartment and results showed that daily leakage for one filter that was tested could be 45m$^3$ (12,000 gal). The test result if extrapolated to operation of all filters meant that nearly 187m$^3$ (50,000 gal)/day of unfiltered water were pumped into the distribution system.

While repairs were underway to seal the joints, *Giardia* samplers were installed on the effluent lines of a repaired filter and another that still had faulty joints. The samplers were installed to test the capability for removal of cysts. The sampler is a yarn-wound orlon filtering device that traps particulate matter including *Giardia* cysts. The sampling results in Table 1 show the difference in operation of the two filters. The most notable difference is the volume of water that was passed through the sampler from the repaired filter as opposed to the one not repaired. At the termination of the sampling period, the sampler on the unrepaired filter was nearly plugged with particulate matter.

## Abatement Procedures

The presence of cysts in finished water with no practical way of controlling or identifying all sources of contamination in the watersheds and the

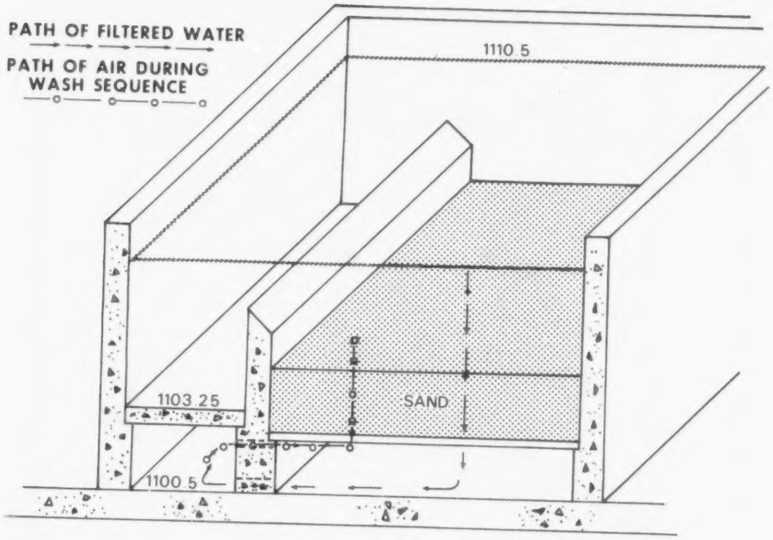

FIG. 2. *Isometric view of the Androscoggin plant filters (not to scale).*

*Table 1. Ammonoosuc plant filter test, Berlin, New Hampshire, 1977*

| | Volume Filtered (Gal) | Turbidity (NTU) | Colonies/100ml |
|---|---|---|---|
| Filter No. 1<br>(Not Repaired) | 285 | 0.23 | 3 (Start)<br><1 (Stop) |
| Filter No. 2<br>(Repaired) | 928 | 0.12 | <1 (Start)<br><1 (Stop) |

uncertainty associated with cyst removal by the Ammonoosuc Plant (and the Androscoggin Plant until repairs were completed) reduced the abatement measures to disinfection and boiling water.

There were a few problems associated with disinfection, the major one being the lack of information on the destructive dose for *Giardia* cysts. In the absence of specific data, concentration-contact time relationships for cysts of *E. histolytica* developed by Chang in 1967 as shown in Figure 3 were used (unpublished data, S.L. Chang). For disinfection to be effective at doses normally applied in water treatment, the contact time requirement is beyond the capability of most treatment plants. Therefore, the dose must be increased to compensate for lack of contact time.

A chlorine contact basin was not incorporated into the design of the Ammonoosuc Plant. The plant effluent was chlorinated and the transmission main between the plant and the distribution system allowed

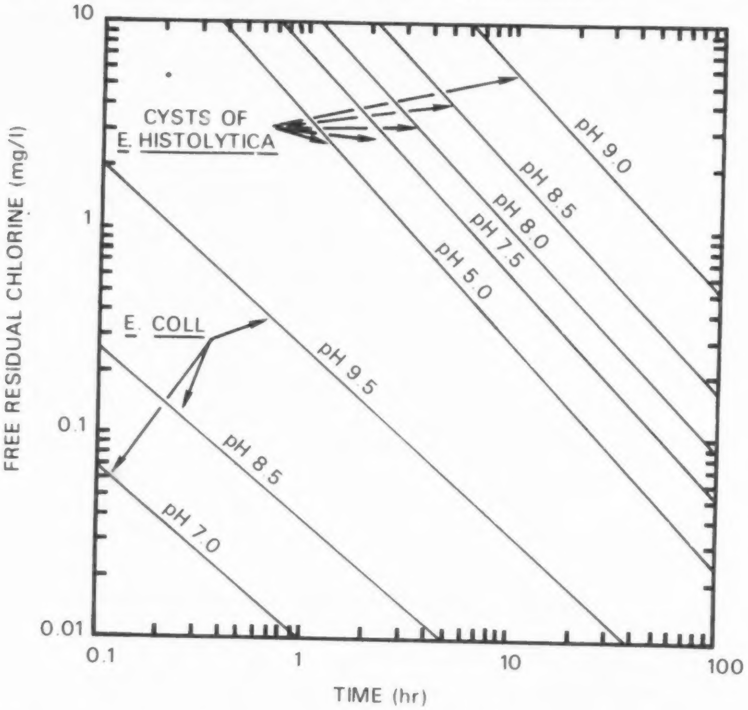

FIG. 3. *Concentration-time relations for 99.999 percent destruction of* E. coli *and cysts of* E. histolytica *by free chlorine at 2 to 5° C (Chang, 1967).*

about one hour of contact time. This required a chlorine residual of 6 mg/l which was temporarily applied until emergency pre-chlorination facilities were installed at Godfrey Dam. The installation at the dam permitted use of 8.8 km (5.5 mi) of transmission main for contact time which reduced chlorine dosage requirements to more acceptable concentrations. The heavy dose of chlorine initially applied at the Ammonoosuc Plant did provide an unexpected benefit in water quality by eliminating a color problem.

Finished water storage facilities were incorporated into the design of the Androscoggin Plant and were used as chlorine contact basins. Capacity of the storage facilities was sufficient to provide about 7 hours of theoretical contact time based on daily plant output. A chlorine residual of 2 mg/l was initially applied; however, a review of a day's operating results showed that the contact time was less than 2 hours due to short-circuiting in the storage facilities. Chlorine feed was then adjusted to meet the requirements shown in Figure 3.

The issuance of a boil-water order is unpopular with water utility management as well as the public. Management resents it because of adverse public reaction and the public does not like to be inconvenienced. A boil-water order was issued in Berlin as a protective measure until repairs and adjustments in treatment were made at the two plants and samples of water were negative for cysts. Unfortunately, the repairs required a longer time than expected and the boil order was in effect for six weeks. The time of boil to destroy cysts was not known so a conservative estimate of 5 minutes was initially recommended. However, there were objections because it was not substantiated by specific data. The local hospital conducted some boiling experiments and found that 15 to 20 minutes were required to rupture the cysts. The recommendation to boil water was then changed from 5 to 20 minutes. The inconvenience of boiling water over a lengthy period of time coupled with rising energy costs did create resentment and some individuals threatened to stop paying their water bills.

## OTHER PROBLEMS AND CONCERNS

A few other problems and concerns deserve discussion. The raw water intake at Godfrey Reservoir was located at the base of the dam. The dam was constructed in the 1930's when the design consideration was to locate the intake at a position which would maximize use of the storage capacity of the reservoir. Current practice requires installation of a number of outlet ports to enable selective withdrawal from several elevations to obtain the best quality of water in the reservoir as conditions change throughout the year. The specific gravity of *Giardia* cysts is greater than water and settling occurs under quiescent conditions of a reservoir. A bottom intake withdraws water from the depths of a reservoir, the area of greatest cyst concentration. This should be avoided if possible for water supply purposes by use of an intake that permits withdrawal from near the reservoir surface.

A concern in water treatment pertains to filtration efficiency during the period immediately following backwash. When a filter is washed and put into operation it takes some time for the bed to stabilize. If effluent turbidity is measured from the beginning of filter operation a spike is noted which generally lasts for about 30 min. Turbidity levels of up to 50% of the turbidity of applied water is measured in filtered water(7). The spike is termed initial breakthrough and, because it normally has no great effect on plant effluent if evaluated in terms of turbidity, it is accepted and no corrective action is taken. It is counteracted in some plants by discharging filtered water to waste during the spiking period. The effect of initial breakthrough is subdued in most plants because multiple filters are discharging to a common effluent manifold and the increase in turbidity from one filter is not sufficient to affect the turbidity level as measured in the total plant output. The concern with filter spike is that it may present the opportune time for passage of cysts and contamination of the water system may occur on an intermittent basis. A filter at the Ammonoosuc Plant was sampled for turbidity after backwashing, and the results shown in Table 2 demonstrate initial breakthrough.

Another concern in water treatment is the disposal of filter backwash and sedimentation basin sludge from plants challenged by *Giardia*. The removal and concentration of cysts by water treatment processes may prevent an

*Table 2. Ammonoosuc plant filter test*
*Berlin, New Hampshire, 1977*

| Filtered Water | | |
|---|---|---|
| Sampling Sequence | | Turbidity (NTU) |
| Prior to backwash | | 0.75 |
| Filter in operation | - Start | 1.55 |
| | - 1 min. | 4.25 |
| | - 2 min. | 2.1 |
| | - 5 min. | 1.5 |
| | - 10 min. | 1.35 |
| | - 15 min. | 1.4 |

outbreak from occurring in one community but the discharge of cyst-laden backwash and sludge may create problems for downstream uses including water supply, recreation and possibly shellfishing. The long-term survival of cysts in water compounds the problem and cyst laden discharges could have far-reaching effects. Also of concern is the discharge of inadequately treated sewage in areas where outbreaks of giardiasis have been notable. Information on wastewater discharges for three states in the northeast is shown in Table 3 (personal communication, P. Karalekas) and indicates a need for concern.

Finally, the heavy doses of chlorine required to destroy cysts create problems with the formation of undesirable treatment by-products. The reaction of certain organic materials with chlorine produces trihalomethanes which are currently under investigation as carcinogens. While chlorination may be providing a solution to an acute health problem in the community, it may be contributing to the occurrence of a chronic situation. While the public naturally reacts to odors and tastes resulting from heavy doses of chlorination, the threat of cancer produces a different reaction. The recommendation to boil water to counteract taste and odor problems has the added benefit of eliminating trihalomethanes. A few minutes of boiling drastically reduces trihalomethane concentrations as shown in Table 4 (personal communication, T. Love). Therefore, boiling

*Table 3. Wastewater discharges*
*Maine, New Hampshire, Vermont, 1978*

| State | Sewage Discharges Raw or Chlorinated Only (No. of communities) | Backwash Water and/or Sludge (No. of plants) | No. of Downstream Water Supplies |
|---|---|---|---|
| Maine | 68 | 12 | 0 |
| New Hampshire | 40 | 2 | 2 |
| Vermont | 27 | 5 | 27* |

* *All these communities use Lake Champlain as a source of water supply.*

*Table 4. Trihalomethane removal by boiling*

| Time of Boiling (min) | Trihalomethane Removal (%) |
|---|---|
| <1 | 10 |
| 1 | 80 |
| 2 | 96 |
| 5 | >99 |

water may be a necessary adjunct to chlorination for protection of public health in some cases.

The problems and concerns presented above are not unique to the Berlin Water System and it is hoped that their discussion will be of benefit to others involved in waterborne outbreaks of giardiasis.

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# An Outbreak of Gastroenteritis Associated with *Giardia Lamblia**

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There is still controversy concerning the pathogenicity of the intestinal flagellate, *Giardia lamblia.* For many years it was generally considered a harmless commensal, because of its presence in many healthy individuals and its lack of tissue invasiveness. Experimental infection of human volunteers resulted in transitory changes in stool pattern in some of the subjects, but not in clinical illness (1). There have been, however, numerous reports of cases of intestinal disturbances associated with the presence of *Giardia,* and without any other detectable cause (2-4). The relief of symptoms in many of these patients after eliminating the giardias with quinacrine has greatly strengthened the case for the pathogenicity of the parasite (5-8).

The manifestations of *Giardia* infection are believed to be due to chronic irritation of the duodenum resulting from the attachment of enormous numbers of trophozoites to the surface of the mucosa (9). The most common symptoms are diarrhea, flatulence and upper abdominal discomfort (3,5,6,8). Steatorrhea is often noted, especially in children (9), who tend to have both a higher carrier rate and a higher rate of symptomatic infection than do adults. The disease is usually sporadic in incidence, though one outbreak involving 44 persons, over half of them children, was reported in England in 1942 (10).

The present report gives the results of an investigation made during an outbreak of gastroenteritis which occurred in Portland, Oregon during the fall and winter of 1954-55. The source, mode of spread, and nature of the etiological agent were never satisfactorily determined. A striking increase in the incidence of *Giardia* in the population at the time of the outbreak and its correlation with cases of diarrheal disease are considered worthy of note, especially since no published reports of similar epidemics could be found.

## EPIDEMIOLOGY AND CLINICAL ASPECTS

The outbreak began in October, 1954 and continued until March, 1955, reaching a peak in late December and early January. The Oregon State

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*This paper was not presented but has been included in the Proceedings at the request of the editors. The manuscript was prepared about 1959 but never published.

Board of Health estimated that at least 50,000 cases occurred during that period. The outbreak appeared at the time to be confined to Portland and the surrounding suburban communities, though later reports made it seem probable that similar outbreaks occurred elsewhere in the state. Age, sex, place of residence or work within the city seemed to play no role in the distribution of cases, except that relatively more cases occurred among adults than among children.

The outbreak was unique in the experience of the city, both in the numbers of people involved and in the character and duration of symptoms. In 500 cases investigated by the epidemiologists, the average duration was 14.8 days, with an extreme range of 1 to 120 days. A third of the cases were of the chronic, intermittent type. Abdominal discomfort, diarrhea, loss of appetite and nausea were the most frequent symptoms. From histories obtained during the course of our investigation, it appeared that a typical individual experience was a series of episodes of diarrhea, occurring at intervals over a period of several weeks. The stools during the acute attacks often were watery, pale and fatty, containing no blood or pus. Though the acute episodes of diarrhea often lasted only a few days at a time, the discomfort, especially in the upper abdomen, the nausea and lack of appetite often persisted between attacks. Most of those affected were able to continue their usual activities; however, those afflicted more severely required hospitalization for uncontrollable diarrhea and marked weight loss.

Neither preceding nor during the outbreak was there any reported increase in the number of infections with salmonella, shigella or amebae. A marked increase in the incidence of *Giardia* found in fecal specimens from patients with diarrhea, especially in those specimens which were liquid, pale and fatty in appearance, was noted by some of the clinical laboratories in October. As the possibility of a relationship between the presence of the flagellate and the current syndrome became known, many physicians began to use quinacrine in treatment. Many of them reported excellent results, with marked improvement in symptoms within 24 to 48 hours, even in cases which previously had been treated unsuccessfully with a variety of other drugs. Others reported equally good results with other methods of treatment. Unfortunately, the number of reports containing parasitological data was too small for any statistical analysis by the epidemiologists. Physicians frequently prescribed quinacrine only on the basis of symptoms, without ordering a stool examination. The lack of clear-cut evidence led the State Board of Health to conclude that the outbreak was caused most likely by an unidentified virus, but the unusual prevalence of *Giardia lamblia* cysts in stools of patients seemed worthy of record.

Because of the strong public interest in the outbreak, the differences of opinion among members of the medical profession concerning the significance of *Giardia* in relation to the disease, and the difficulty of obtaining adequate data, an investigation was conducted among a group of people from whom reasonably complete records could be obtained. The study was initiated in January, 1955, at a time when the outbreak was declining.

## PROCEDURES

The group chosen for the survey consisted of 81 persons, 68 men and 13 women, all of them students or employees of the Medical School. Twenty-nine other individuals were also studied, but not included in the survey group. Most of them were students or employees who were suffering from gastroenteritis, and the others were members of the families of those found to be harboring *Giardia*.

Each member of the survey group submitted up to 3 stool specimens for examination, unless *Giardia* was found in one of the specimens. Those found to be harboring the parasite were not required to submit further specimens. The number of examinations performed on the patient-contact group varied widely, but usually consisted of only 1 specimen. A few patients, in all of whom *Giardia* had been found on the first examination, submitted several specimens over a period of weeks. Each person examined filled out a questionnaire, stating whether he had had a gastrointestinal disturbance of any kind during the preceding 6 months, the nature of the symptoms, duration, type of treatment, and the results of previous stool examinations.

Fresh, unpreserved specimens were used, and all were examined in iodine-stained wet mounts after concentration by the formalin-ether technic. Those specimens which were of softer than normal consistency were examined in saline and iodine mounts before concentration. All soft specimens, as well as those found to contain *Giardia* and all those from individuals with a history of present or recent gastrointestinal illness, were also examined for bacterial enteric pathogens and the serological 0 group of 10 *Escherichia coli* colonies was determined. Iron hematoxylin stains were made from 20 of the specimens which contained large numbers of *Giardia*.

## RESULTS

The bacteriological studies revealed no enteric pathogens, and no pattern of *E. coli* grouping appeared. The incidence of intestinal protozoa other than *Giardia* did not differ from that found in similar groups in the past.

Of the survey group, 37% (30 of 81) were found to be harboring *Giardia*. Forty-two percent of the survey group gave a history of a gastrointestinal disturbance within the previous 6 months, and 21% were having symptoms of diarrhea, flatulence, abdominal pain or discomfort at the time the examinations were made. Since no significant difference was found in the number of positive results in those without symptoms and those who had recovered from previous symptoms before the examination was made, only those who were symptomatic at the time of examination will be considered separately. Table 1 shows the comparative incidence of *Giardia* in the two groups. Included for comparison are the results obtained from 112 students and employees examined at other times. Fifty of these 112 persons were studied prior to the epidemic year, most of them having had 5 specimens examined. The remainder were students in the class following the one examined during the outbreak and were studied at the same time the following year. Procedures for the examination of specimens were the same for all groups.

*Table 1. Comparative incidence of* Giardia lamblia *during epidemic and non-epidemic periods.*

| | Epidemic period | | | Non-epidemic period | | |
|---|---|---|---|---|---|---|
| | Gastro-intestinal symptoms present | Gastro-intestinal symptoms absent | Total | Gastro-intestinal symptoms present | Gastro-intestinal symptoms absent | Total |
| *Giardia* + | 15 | 15 | 30 | 0 | 8 | 8 |
| *Giardia* − | 2 | 49 | 51 | 2 | 102 | 104 |
| Total | 17 | 64 | 81 | 2 | 110 | 112 |

Statistical analysis of the results showed that, in spite of the fact that only half of those harboring *Giardia* were having symptoms during non-epidemic periods, a highly significant association existed between the presence of *Giardia* and symptoms during the epidemic period. The Chi square for the association was 21.5, as compared with an expected 3.184 for a chance probability of 0.05.

All but 5 of the 29 persons in the patient-contact group were having symptoms at the time of examination. *Giardia* was found in 2 of the 5 normal persons and in 16 of the 24 patients. Those with negative findings were examined only once. Since one of the characteristics of the outbreak was the chronicity of the symptoms and since several of those with symptoms were examined during or immediately after a short, acute attack of diarrhea which subsided spontaneously within a day or two, a comparison was made of all those who had symptoms, both among the survey group and the patients, based on the duration of illness. To simplify the analysis, an arbitrary division was made based on duration of more or less than one week. The results are shown in Table 2.

Of the 3 people in the "chronic" series who were negative, 1 was a baby whose diarrhea followed a series of immunizing injections, and a second was an adult who developed diarrhea after intensive antibiotic therapy for an infection. None of the 3 was examined more than once. All of the people who were having symptoms characteristic of the outbreak, and from whom at least 3 specimens were obtained, were found to be excreting *Giardia*, usually in enormous numbers. Twelve of them received quinacrine after the parasites were found and all reported prompt and complete relief of their symptoms, which in some of the cases had been present for 2 months. At

*Table 2. Relation between duration of symptoms and presence of* Giardia *in 41 cases of gastroenteritis*

| Duration of symptoms | No. of persons | No. positive for *Giardia* | % positive |
|---|---|---|---|
| More than 1 week | 31 | 28 | 90 |
| Less than 1 week | 10 | 3 | 30 |

least 7 of the 12 relapsed shortly after discontinuing therapy. In 2 of those, the only ones that were studied adequately, the flagellates were not demonstrable during the symptom-free period, but were found in large numbers during relapses. Two others, who were not examined again, reported relapses and responded promptly to quinacrine treatment.

The characteristics of the onset of symptoms were reported in 26 of the 28 chronic cases in which parasites were found. In only one of these was the onset described as abrupt. The 25 others described onset as gradual or insidious, with symptoms increasing in severity over a period of days or weeks, or as maintaining from the beginning a fairly uniform, low-grade course.

Only 14 people in the entire series were examined within 5 days of onset of their first symptoms. Half of them were negative for *Giardia*. Two of these patients were babies with mild to moderate diarrhea. Three of the adults had attacks lasting only a few hours, with nausea and vomiting as prominent symptoms. The others had attacks lasting 2 to 3 days. In all, symptoms subsided without specific treatment and without known recurrences.

Three of the 7 patients with *Giardia* also had brief attacks, without specific therapy or known recurrences, though 2 were still excreting the parasites when re-examined a month later. The other 4 had a more prolonged course. One recovered spontaneously after 9 days, though his stools still contained *Giardia* on re-examination 6 weeks after recovery. Two had typical severe, chronic cases, which responded promptly when quinacrine was finally administered, but both later had at least 2 relapses. The fourth experienced mild symptoms of abdominal discomfort and soft stools for at least a week following a severe attack of diarrhea, but was not examined again.

In none of our cases was it possible to demonstrate to our complete satisfaction that the patient was parasite-free until the onset of symptoms, and developed chronic disease with the establishment of *Giardia* infection. The patient who most nearly fulfilled the requirements had undergone numerous parasitological examinations in the past, none of them positive, and had large numbers of *Giardia* on the first examination following the onset of symptoms. The disease in this case was typical in the nature of symptoms, long duration and response to therapy. The last negative examination, however, had been made several months before the attack. Another reported having had slight diarrhea and minor malaise during the second week in January, the symptoms subsiding without treatment. Three stool specimens, examined between January 24 and February 2, were negative for *Giardia*. Diarrhea and intestinal discomfort reappeared on March 4, after a course of sulfonamides for a sinus infection. A stool specimen examined on March 9, while the diarrhea was still severe, contained enormous numbers of *Giardia*. The symptoms ceased within 24 hours after the administration of quinacrine. Both of these cases strongly suggest the association between the parasites and the diarrheal attacks, but neither provides proof of the association.

## DISCUSSION

The results of this investigation, and a comparison of these results with those of other periods, make it clear that there was an entirely abnormal incidence of *Giardia* infection in the group studied at the time of the outbreak. The flagellate was found in 44% of those studied during the outbreak, in contrast to 7% of those examined during non-epidemic periods. Even if only those who gave no history of present or past intestinal disturbance are considered, the rate of infection was 24%, or over 3 times that of the control figure.

It is apparent that the presence of the parasite did not necessarily result in disease, since half of those infected were asymptomatic. This is in accordance with the observations of other workers (3). The role of *Giardia* in the transitory cases, either mild or acute, is difficult to assess, because of the small numbers which were studied and the high carrier rate that existed at the time of the study. Undoubtedly many, and very possibly all, of the acute, brief episodes of vomiting and diarrhea were due to other causes, since they were similar in symptomatology and course to cases which occur sporadically every winter. It does not appear from our data that *Giardia* played any role of consequence in these. Its association with the several mild cases of transitory abdominal discomfort and a few unwontedly soft stools is also undetermined, especially since its continued presence did not lead to continued symptoms.

The high rate of repeated relapses among the quinacrine-treated cases is contrary to the usual experience, and to the experience here with cases which have occurred sporadically since the outbreak. It is quite possible that the apparent relapses were actually reinfections, the people acquiring them being highly susceptible and the possibilities for reinfection being extremely great at the time.

We feel that our results do give support to the belief that *Giardia* was responsible for the chronic syndrome which was the outstanding feature of the outbreak. This belief is based on the constant presence of the parasites in those suffering from this syndrome, the uniformity of their symptoms, the favorable effect of quinacrine, and the similarity of the symptoms to those described by others as characteristic of giardiasis. The infrequency with which acute attacks were recorded as preceding or initiating the symptoms in the chronic cases suggest that the enhanced pathogenicity of the parasite in these cases was not due to the action of another acute infection in preparing the way for its establishment and unrestrained multiplication. We have no adequate explanation for the extreme variability in the results of *Giardia* infection in different individuals, nor for the circumstances which led to its exceptional prevalence during this period. We doubt, however, that the experience here was unique, and suggest that giardiasis be more seriously considered than it usually is both in sporadic cases in adults and in outbreaks in which the symptomatology is similar to that described here.

## SUMMARY

An outbreak of gastroenteritis, characterized by the exceptionally prolonged course of many of the cases, is described. A survey made during the outbreak revealed that only *Giardia lamblia*, of the known possible etiological agents of such a syndrome, was detectable in the majority of those affected. The results of the survey are presented and discussed, and the reasons given for the conclusion that the prolonged attacks were probably chiefly due to *Giardia* infection.

(*Editors Note:* Supplementary information developed by the Oregon State Board of Health and supplied by the author follows the References).

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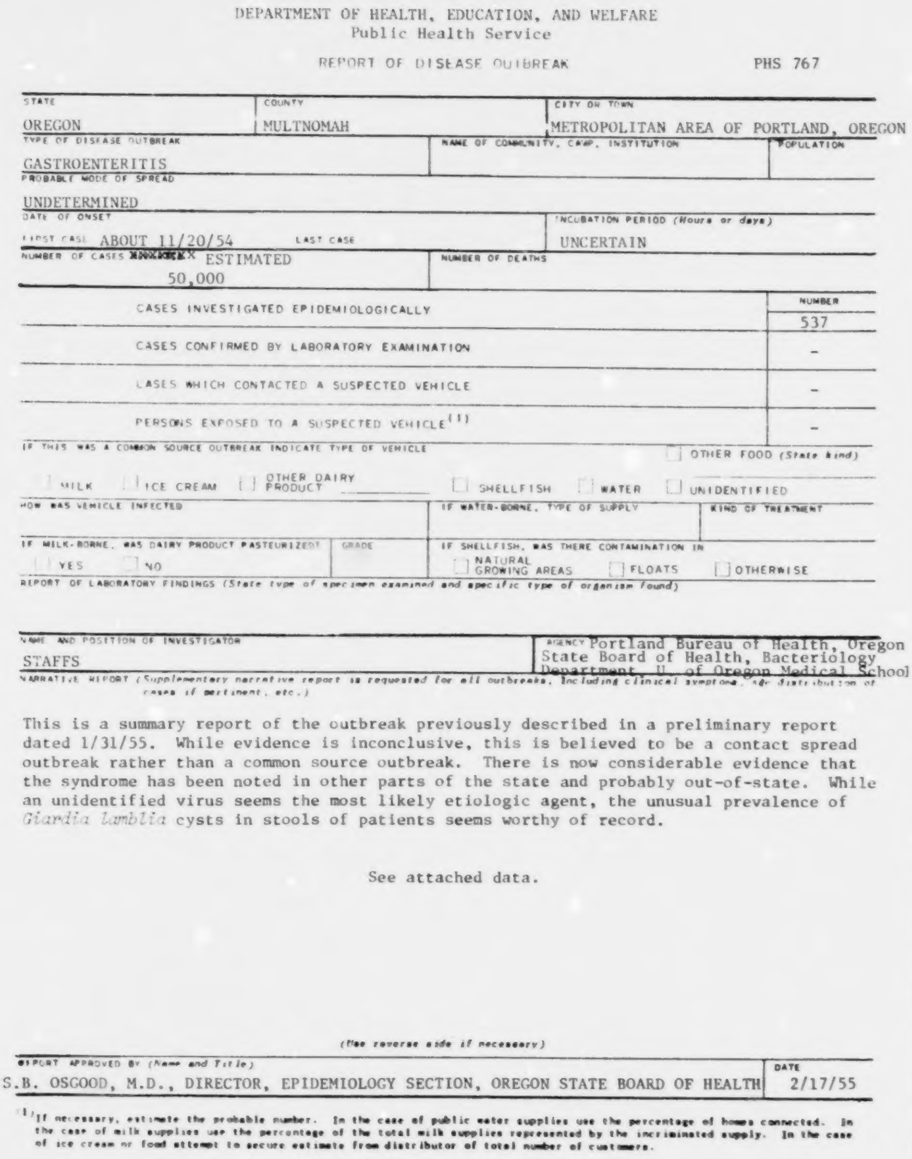


DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

REPORT OF DISEASE OUTBREAK PHS 767

STATE: OREGON
COUNTY: MULTNOMAH
CITY OR TOWN: METROPOLITAN AREA OF PORTLAND, OREGON
TYPE OF DISEASE OUTBREAK: GASTROENTERITIS
NAME OF COMMUNITY, CAMP, INSTITUTION:
POPULATION:
PROBABLE MODE OF SPREAD: UNDETERMINED
DATE OF ONSET: FIRST CASE ABOUT 11/20/54 LAST CASE
INCUBATION PERIOD (Hours or days): UNCERTAIN
NUMBER OF CASES: ESTIMATED 50,000
NUMBER OF DEATHS:

| | NUMBER |
|---|---|
| CASES INVESTIGATED EPIDEMIOLOGICALLY | 537 |
| CASES CONFIRMED BY LABORATORY EXAMINATION | - |
| CASES WHICH CONTACTED A SUSPECTED VEHICLE | - |
| PERSONS EXPOSED TO A SUSPECTED VEHICLE[1] | - |

IF THIS WAS A COMMON SOURCE OUTBREAK INDICATE TYPE OF VEHICLE
☐ MILK ☐ ICE CREAM ☐ OTHER DAIRY PRODUCT ______ ☐ SHELLFISH ☐ WATER ☐ OTHER FOOD (State kind) ☐ UNIDENTIFIED

HOW WAS VEHICLE INFECTED:
IF WATER-BORNE, TYPE OF SUPPLY:
KIND OF TREATMENT:
IF MILK-BORNE, WAS DAIRY PRODUCT PASTEURIZED? ☐ YES ☐ NO
GRADE:
IF SHELLFISH, WAS THERE CONTAMINATION IN ☐ NATURAL GROWING AREAS ☐ FLOATS ☐ OTHERWISE
REPORT OF LABORATORY FINDINGS (State type of specimen examined and specific type of organism found)

NAME AND POSITION OF INVESTIGATOR: STAFFS
AGENCY: Portland Bureau of Health, Oregon State Board of Health, Bacteriology Department, U. of Oregon Medical School

NARRATIVE REPORT (Supplementary narrative report is requested for all outbreaks, including clinical symptoms, age distribution of cases if pertinent, etc.)

This is a summary report of the outbreak previously described in a preliminary report dated 1/31/55. While evidence is inconclusive, this is believed to be a contact spread outbreak rather than a common source outbreak. There is now considerable evidence that the syndrome has been noted in other parts of the state and probably out-of-state. While an unidentified virus seems the most likely etiologic agent, the unusual prevalence of *Giardia lamblia* cysts in stools of patients seems worthy of record.

See attached data.

(Use reverse side if necessary)

REPORT APPROVED BY (Name and Title): S.B. OSGOOD, M.D., DIRECTOR, EPIDEMIOLOGY SECTION, OREGON STATE BOARD OF HEALTH
DATE: 2/17/55

(1) If necessary, estimate the probable number. In the case of public water supplies use the percentage of homes connected. In the case of milk supplies use the percentage of the total milk supplies represented by the incriminated supply. In the case of ice cream or food attempt to secure estimate from distributor of total number of customers.

## RESULTS OF UNIVERSITY OF OREGON MEDICAL SCHOOL STUDY ON GASTROENTERITIS OUTBREAK

* * * * * * * * * * * * *     * * * * * * * * * * * * *

A. Virus Isolation

No virus isolated from stools of acute cases as of 2/15/55.

B. Enteric Pathogens (Single stool from 100 persons, usual cultural technics).

No enteric pathogens isolated as of 2/2/55.

C. Intestinal Parasites

I. Specimens obtained from 23 patients with acute gastroenteritis, 68 students in one class of medical students and 9 members of bacteriology department staff for a total of 100 cases.

II. Methods

All stools examined by a concentration method for parasites and ova; when initial stool was negative, up to 3 specimens were secured on the same case before any were called negative for *Giardia*.

Histories were obtained from the "survey" group of 77 cases to determine whether or not there was any history of gastrointestinal symptoms during the prior 3 months.

III. Results

A. No *E. histolytica* and very few intestinal parasites other than *Giardia* were found.

B. *Giardia* were found in 15 of 23 (65%) of the "Patient" group and in 25 of 77 (32.5%) of the "survey" group for an overall percentage of 40% positive *Giardia*. This contrasts with less than 3% positive for *Giardia* in a similar but smaller group of medical students examined by the same technic about 1 year ago.

C. Of the 25 in the survey group positive for *Giardia* 16 (64%) had gastrointestinal symptoms (10 prolonged or recurrent and 2 relieved by atabrine) whereas 9 (36%) denied any symptoms and in 4 of these *Giardia* were numerous.

D. *Giardia lamblia* (chiefly cysts) were found in 31 (58%) of the group that had symptoms and in 9 (20%) of the group that had no symptoms.

/dl Epid Section-OSBH
2/17/55

## RESULTS OF LABORATORY POLL ON STOOL SPECIMENS EXAMINED FOR PARASITES

* * * * * * * * * * * * * * * * * * *

| Name of Laboratory | OCTOBER 1954 | | | NOVEMBER 1954 | | | DECEMBER 1954 | | | JANUARY 1955 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Spec. Examined | No. *Giardia* + | % *Giardia* + | No. Spec. Examined | No. *Giardia* + | % *Giardia* + | No. Spec. Examined | No. *Giardia* + | % *Giardia* + | No. Spec. Examined | No *Giardia* + | % *Giardia* + |
| OSBH HYGIENIC LABORATORY | 38 | 2 | 5.2 | 14 | - | 0.0 | 33 | 9 | 27 | 53 | 14 | 26 |
| U. OF O. OUT-PATIENT CLINIC (Dr. Grondahl) | - | - | - | 49 | 1 | 0.5 | 57 | 3 | 5.3 | 165 | 24 | 14 |
| GOOD SAMARITAN HOSPITAL | - | - | - | 16 | - | 0.0 | 52 | 11 | 22 | 104 | 26 | 25 |
| ST. VINCENTS HOSPITAL | - | - | - | 10 | 2 | 20 | 5 | 3 | 60 | 27 | 1 | 3.7 |
| PHYSICIAN'S MEDICAL (Dr. Crynes) | - | - | - | - | - | - | 30 | 21 | 70 | - | - | - |
| DITTEBRANDT LABORATORIES | - | - | - | 14 | 1 | 7 | 18 | 6 | 33 | 62 | 21 | 33 |
| MANLOVE LAB-ORATORIES | - | - | - | - | - | - | 30 | 1 | 0.3 | 14 | 3 | 21 |
| JACKSON TOWER (Dr. Minckler) | - | - | - | - | - | - | 9 | 4 | 44 | 16 | 6 | 37 |
| PROVIDENCE HOSPITAL | - | - | - | 15 | - | 0 | 30 | - | 0 | - | - | - |
| TOTAL | 38 | 2 | 5.2% | 118 | 4 | 3.4% | 264 | 58 | 22% | 441 | 95 | 21.5% |
| | (1 laboratory reporting) | | | (7 laboratories) | | | (9 laboratories) | | | (7 laboratories) | | |

/dl Epid Section-OSBH
2/17/55

## RESULTS OF HETEROPHILE ANTIBODY TESTS AND SEROLOGIC TESTS FOR SYPHILIS ON KNOWN GASTROENTERITIS CASES

* * * * * * * * * * * * * * * *

A. HETEROPHILE ANTIBODY TESTS

1 case, *Giardia* present, antibody titer 1/80 two weeks after onset and 1/40 three weeks after onset.

B. SEROLOGIC TESTS FOR SYPHILIS

17 cases, *Giardia* known to be present in stools of 11, STS negative on all 17 cases - blood taken 7 to 35 days after onset.

(a few doctors had reported doubtful serologic tests for syphilis and slight reactions to the heterophile antibody test on 1 or 2 cases of the current gastroenteritis syndrome)

/dl 2/17/55

## DIARRHEAL ILLNESS

Total Tabulation Including Cases From Private Doctors, OSBH Cases, Plus State Office Building Cases

I. Multiple vs Single Case Households

A. Total Household - 253
B. Single Case Household - 127 (50%)
C. Multiple Case Household - 126 (50%)

II. Duration of Illness

A. Private M.D. Cases (142 cases)
Range - 1 - 120 days
Average - 18 days

B. OSBH (42 cases)
Range - 30 days
Average - 8 days

C. State Office Building (317 cases)
Range - 1 - 90 days
Average - 14.3 days

D. Total (501 cases)
Range - 1 - 120 days
Average - 14.8 days

E. Chronic, intermittent type illness

1. Private M.D. - 52 of 142 cases (36.6%)
2. OSBH - 9 of 42 cases (21.4%)
3. State Office Building - 105 of 317 cases (33.1%)

III. Sex Distinction (537 cases)

A. Male - 217 (40.5%)
B. Female - 320 (59.5%)

IV. Age Distribution (461 cases)

A. Cases

Less than 5 - 27 ( 5.86%)
5 - 9 - 28 ( 6.1%)
10 - 14 - 27 ( 5.86%)
15 - 19 - 23 ( 5.0%)
20 and over - 356 (77.0%)

(Age distribution of Portland population, 1950 census for comparison)

Under 5 - 9.2%
5 - 9 - 6.8%
10 - 14 - 5.4%
15 - 19 - 5.1%
20 and over - 73.4%

B. Exposures (740 total)

Less than 5 - 85 (11.5%)
5 - 9 - 72 ( 9.7%)
10 - 14 - 41 ( 5.5%)
15 - 19 - 44 ( 5.9%)
20 and over - 498 ( 67%)

C. Over-all attack rate in exposed household

1. Total exposed - 740
2. Total ill - 461
3. Rate - 62%

D. Age-specific attack rate

Less than 5 - 31.7%
5 - 9 - 39%
10 - 14 - 66%
15 - 19 - 52%
20 and over - 71.5%

V. Symptoms (358 cases)

Loss appetite - 246 ( 69%)

Nausea - 238 ( 66%)

Vomiting - 129 (36.0%)

Abdominal discomfort - 291 ( 81%)

Diarrhea - 275 ( 77%)

Muscle Pain - 101 (28.3%)

Fever - 92 (25.7%)

Headache - 152 (42.5%)

Cough - 62 (17.3%)

Sore Throat - 52 (14.5%)

Runny Nose - 51 (14.6%)

VI. Duration of Illness vs Therapy

I. Atabrine therapy (71 cases)

A. Average duration before treatment - 11.1 days

B. Average duration after treatment - 7.8 days

C. Average duration total illness - 18.9 days

II. Treated by means other than atabrine (103 cases)

A. Average duration before treatment - 5.8 days

B. Average duration after treatment - 9.7 days

C. Average duration total illness - 15.5 days

## REPORT ON DIARRHEAL DISEASE IN STATE OFFICE EMPLOYEES

I. Attack rate among employees

A. 561 persons reported on
B. 173 persons ill
C. Attack rate - 30.8%

II. Duration of illness (known on 317 cases)

A. Average duration - 14.3 days
B. Range - 1 to 90 days
C. Chronic relapsing type of illness - 105 cases (33.3%)

III. Multiple vs Single Household cases (182 households)

A. Single Case Household - 95 (52%)
B. Multiple Case Household - 87 (48%)

2 cases - 53
3 cases - 18
4 cases - 14
5 cases - 1

IV. Sex Distribution (323 cases)

A. Male - 132 (41%)
B. Female - 191 (59%)

V. Age Distribution

A. Cases - 319

Less than 5 - 11 ( 3.45%)
5 - 9 - 13 ( 4.1%)
10 - 14 - 23 ( 7.2%)
15 - 19 - 20 ( 6.3%)
20 and over - 252 (79.0%)

B. Exposures - 485

Less than 5 - 47 (9.7%)
5 - 9 - 33 (6.8%)
10 - 14 - 27 (5.6%)
15 - 19 - 37 (7.6%)
20 and over - 341 ( 70%)

C. Over-all attack rate in exposed household

1. Total exposed - 485
2. Ill - 319
3. Rate - 66%

D. Age-specific attack rates

Less than 5 - 23.4%
5 - 9 - 39.4%
10 - 14 - 85%
15 - 19 - 54%
20 and over - 74%

VI. Treatment (known on 184 cases)

A. Non-specific (none, Pepto Bismol, etc.) - 108 (58.6%)

| | | | |
|---|---|---|---|
| B. | Bismuth and Paregoric | 10 | 37 (20%) |
| C. | Kaopectate | 22 | |
| D. | Antispasmodics | 5 | |
| E. | "Specific" therapy | 40 (21.7%) | |

Aureomycin - 3
Creomycin - 2
Penicillin - 7
Terramycin - 2
Streptomycin - 1
Ilotycin - 1
Dramamine - 1
Sulfa - 1
Antihistamine - 3
Atabrine - 6
Unknown prescription - 13

VII. Symptoms (174 cases)

Loss of appetite - 119 ( 68%)
Nausea - 122 ( 70%)
Vomiting - 58 ( 33%)
Abdominal Discomfort - 154 (88.5%)
Diarrhea - 131 ( 75%)
Muscle Pain - 67 (38.5%)
Fever - 63 ( 36%)
Headache - 112 (64.4%)
Cough - 35 ( 20%)
Sore Throat - 45 ( 26%)
Runny Nose - 43 (24.7%)

February 16, 1955

## ABSENTEEISM DATA IN RELATION TO PORTLAND GASTROENTERITIS OUTBREAK

I. City of Portland (Municipal employees, 3604 employed as of 1/1/55, absentees visited by public health nurses)

| Year | Month | Total Absenteeism | Absenteeism due to "flu" and "diarrhea" |
|---|---|---|---|
| 1953 | November | 667 | 187 |
| 1954 | November | 561 | 161 |
| 1953 | December | 664 | 142 |
| 1954 | December | 750 | 241 |
| 1954 | January | 616 | 145 |
| 1955 | January | 870 | 476 (Est. as 159 "flu", 317 "diarrhea") |
| 1955 | February (1st 2 weeks) | 355 | 83 (37 "flu", 46 "diarrhea") |

II. Portland Public Schools--Days Absent, all causes

| Year | Month | Absenteeism in pupil days |
|---|---|---|
| 1953 | November | 88,951 |
| 1954 | November | 70,622 |
| 1953 | December | 95,074 |
| 1954 | December | 83,295 |
| 1954 | January | |
| 1955 | January | |

III. Portland General Electric Company--Absenteeism all causes

| Year | Month | Man days lost | Month | Man days lost | Month | Man days lost | Month | Man days lost |
|---|---|---|---|---|---|---|---|---|
| 1953 | Oct. | 192 | Nov. | 204 | Dec. | 227 | Jan. | 194 |
| 1954 | Oct. | 200 | Nov. | 221 | Dec. | 266 | Jan. | 277 |
| 1955 | | | | | | | Jan. | 318 |

(December 1954, 119 specified as "flu" and 3 as "enteritis", January 1955, 139 "flu" and 7 as "enteritis)

IV. Portland Gas and Coke--Absenteeism in Man Hours

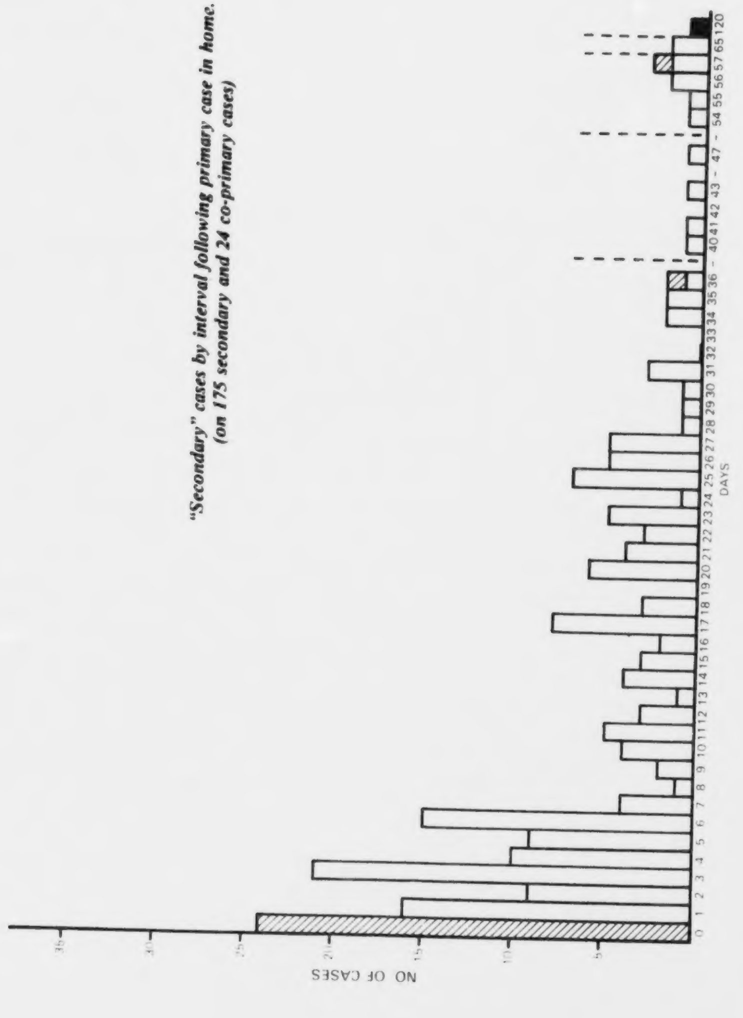
| | Year | Month | Man hrs. lost | Month | Man hrs. lost | Month | Man hrs. lost | Month | Man hrs. lost |
|---|---|---|---|---|---|---|---|---|---|
| (919 employed) | 1953 | Oct. | 1944 | Nov. | 3841 | Dec. | 3748 | Jan. | 2488 |
| (818 employed) | 1954 | Oct. | 2585 | Nov. | 2972 | Dec. | 3504 | Jan. | 3064 |
| | 1955 | | | | | | | Jan. | 2976 |

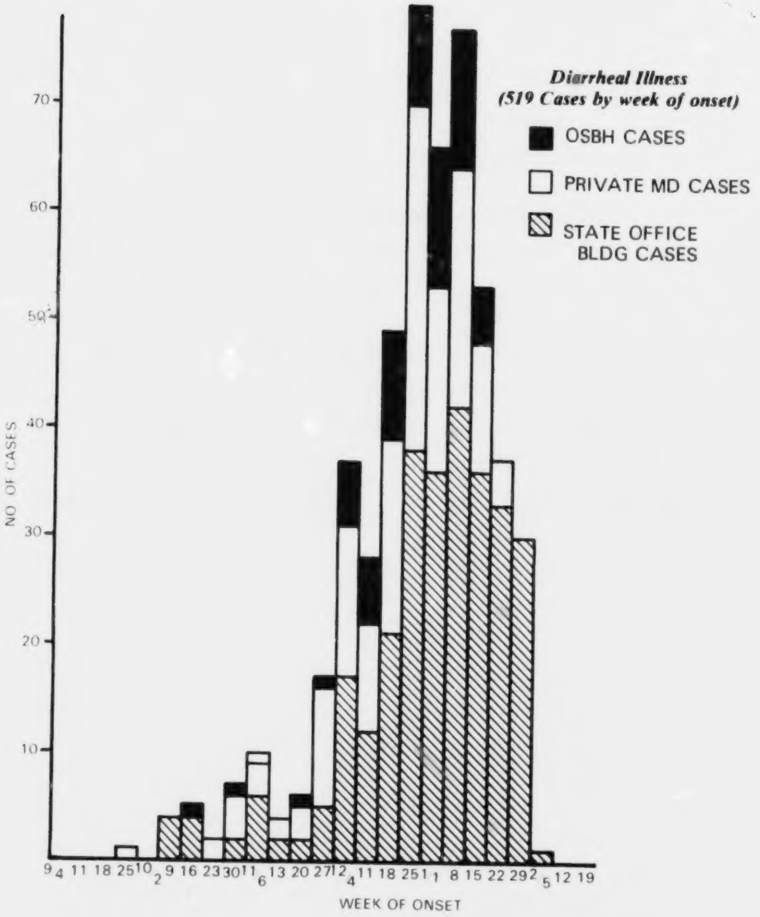
V. National Biscuit Company--Absenteeism, all causes (Year of 1954 - 2.64%)

1954 January 2.61% December 1.96%
1955 January 1st week 2.46%, 2nd week 2.45%, 3rd week 2.65%, 4th week 2.76%

/dl Epid Section-OSBH
2/17/55

*"Secondary" cases by interval following primary case in home.*
*(on 175 secondary and 24 co-primary cases)*




*Diarrheal Illness*
*(519 Cases by week of onset)*
■ OSBH CASES
☐ PRIVATE MD CASES
▨ STATE OFFICE BLDG CASES
NO OF CASES
70
60
50
40
30
20
10
9 4 11 18 25 10 2 9 16 23 30 11 6 13 20 27 12 4 11 18 25 1 1 8 15 22 29 2 5 12 19
WEEK OF ONSET

# SESSION IV - DETECTION METHODOLOGY

*Chairman - John C. Hoff,*
*U.S. EPA, MERL, Cincinnati, Ohio*

## Methods for Detection of *Giardia* Cysts in Water Supplies

**W. Jakubowski, T. H. Ericksen**

## The Propagation of *Giardia* Trophozoites In Vitro

**E. A. Meyer**

## Induction of *Giardia* Excystation and the Effect of Temperature on Cyst Viability as Compared by Eosin-Exclusion and In Vitro Excystation

**A. K. Bingham, E. L. Jarroll, Jr., E. A. Meyer, S. Radulescu**

# Methods for Detection of *Giardia* Cysts in Water Supplies

**Walter Jakubowski and T. H. Ericksen**
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*Health Effects Research Laboratory*
*Field Studies Division*
*Epidemiology Branch*
*Bacterial and Parasitic Diseases Group*
*Cincinnati, Ohio*

## ABSTRACT

The development and application of methods for detecting *Giardia* cysts in water are reviewed. The concentration of cysts in raw sewage was calculated to be between $9.6 \times 10^3$ and $2.4 \times 10^5$ cysts/l when 1-25% of the population is infected. Evaluation and application of a large volume sample collection and processing method are described. A step-by-step analysis of the procedure indicates 42% of cysts lost in preparing a homogenate of the primary Orlon filter, 58 to 71% loss of cysts when using a 7 μm mesh nylon screening process, and more than 90% loss if flocculation is used to clarify the homogenate. Suggested modifications for producing a fourfold increase in recovery efficiency are described and evaluated. Sampling considerations, including sample volume, frequency, flow rate, storage, shipment, processing, and interpretation of results are discussed. A sample volume of 380 l (100 gal) is suggested for occurrence surveys in water supplies when concurrent epidemiologic and/or watershed animal surveys are performed.

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The detection, identification, and enumeration of microbiological contaminants, and in the present instance specifically *Giardia* cysts in water supplies, is of concern to workers in a number of different disciplines. The microbiologist and the epidemiologist want to know the source of contamination, the level of contamination, the relationship to indicator organisms, and the reservoirs of infection. The engineer and the utility operator want to know how to remove or inactivate *Giardia* by water treatment. Those responsible for administering drinking water programs want to know where and when the organism occurs in water, the suitability and availability of monitoring methods, and whether or not maximum contaminant levels or treatment requirements should be promulgated.

These data are required on this pathogen because recent outbreaks, in particular those in Camas, Washington(1, 2) and Berlin, New Hampshire(3), have demonstrated that the coliform standard cannot be relied upon to indicate the safety of a water supply with respect to *Giardia*. The possibility that other organisms, such as yeasts, could act as alternate indicators of *Giardia* contamination remains to be investigated. The application of cultural techniques to provide answers to the questions being posed is not now possible for *Giardia* in drinking water. In addition, certain basic information (e.g., how to determine species of origin, viability, or infectivity) necessary for the interpretation of results obtained through microscopic examination is lacking. The purpose of this paper is to describe the state-of-the-art with regard to sampling methodology for the detection, identification, and enumeration of *Giardia* cysts in water supplies.

## DETECTION METHODS

At the present time, there is no cultural method for detecting *Giardia* cysts in water samples. However, Meyer(4,5) and others have successfully cultivated the trophozoite stage, and the recent development of an excystation technique(6) may subsequently lead the way to development of cultural methods. The available alternatives are animal feeding and direct microscopic examination. Animal feeding experiments require special handling and isolation facilities, and are time-consuming and expensive. Their application to surveys and monitoring would, therefore, be limited by economic and practical considerations. Animal feeding, however, is the only method for demonstrating the infectivity of *Giardia* cysts.

Microscopic methods of detection are not quite as time-consuming as animal feeding but they are tedious. In addition, identification of the organism is dependent upon the training, experience, skill, and patience of the examiner. The results of a proficiency-testing program reported(7) by the Center for Disease Control (CDC) indicated that between 20% and 35% of public and private laboratories could not correctly identify intestinal protozoa. Furthermore, the identification of protozoan cysts is not a skill that would be available in most water supply microbiological laboratories. In performing microscopic analysis it is also important to have a clean preparation as free as possible of inorganic and organic debris that could mask the presence of the organisms or interfere with identification. Nevertheless, recognizing these limitations, microscopic methods are the only currently feasible approach for detection of *Giardia* in water samples.

## THEORETICAL LEVELS OF CYSTS IN SEWAGE AND WATER

In developing methods, it would be advantageous to have some information on cyst levels that might be expected in sewage and water samples. *Giardia* is an intestinal parasite not likely to multiply in a water environment outside of a host. Tsuchiya(8) studied cysts produced by two human carriers, A and B, for 36 and 28 days, respectively. An analysis of these data indicated a mean of $1.1 \times 10^6$ cysts/g of stool for subject A with a maximum observed level of $3.3 \times 10^6$ cysts/g. Mean daily production of cysts for subject A was $2.1 \times 10^8$ with a maximum of $7.1 \times 10^8$. Results for subject B were similar (mean of $1.6 \times 10^6$/g with a maximum of $4.3 \times 10^6$, and daily production of $2.0 \times 10^8$ with a maximum of $6.4 \times 10^8$). Tsuchiya did not report the subjects' ages but it is assumed that they were adults.

Danciger and Lopez(9) studied cyst production in 15 children 3 to 7 years of age. Three patterns of excretion were noted: high, with large numbers of cysts in nearly all stools; low, with small numbers of cysts in 40% of the stools; and mixed, where periods of high excretion alternated with shorter periods of low excretion and an average of about 60% positive stools. The number of cysts excreted ranged up to $2.2 \times 10^6$/g of formed stool with an overall mean of $5.8 \times 10^5$/g. Total daily production of cysts on a per capita basis was not reported.

The per capita daily usage of water from public supplies in the U. S. in 1975 was 638 l (168 gal)(10). Water lost in the distribution systems and used for public activities such as firefighting, street cleaning, etc. was estimated at 36% or 230 l (60 gal). Industrial and commercial use was 31% or 198 l (52 gal). This results in an estimated residential usage of 210 l (55 gal)/person/day. Assuming that the average daily water usage for a family of four is 840 l (220 gal)/day, and that $2.0 \times 10^8$ cysts are shed daily by an infected adult, the theoretical concentration of cysts in raw sewage would be $2.4 \times 10^5$ cysts/l if one of the individuals (25%) was shedding cysts. If 1% of the population were infected, the concentration of cysts in raw sewage would be $9.6 \times 10^3$ cysts/l. These calculations are based upon a uniform dispersion of fecal material in sewage originating entirely from domestic sources. Calculation of cyst levels in water supplies would then be dependent upon the degree of raw domestic sewage contamination of a given water supply. Various surveys have indicated the prevalence of *Giardia*-infected individuals to be in the range of 1 to 24%(11).

The Camas, Washington outbreak(1) and reports of infections in campers and hikers(12) suggest that water supplies may become contaminated with *Giardia* by sources other than human fecal discharge. Beavers were implicated in the Camas outbreak and the extent to which wild and domestic animals may act as reservoirs of infection is currently being determined(13). Sparkling mountain streams in pristine locales may be subject to contamination by wild animals. The role of man in disseminating infection in wild animals needs to be determined by comparing infection rates in animals in those areas open and closed to human use. In a survey conducted in Washington State over the last 2 years (14), 7% of beaver and 33% of muskrat examined were *Giardia*-positive. It was noted that many of the muskrats were trapped close to human dwellings and in areas which may have received human sewage contamination.

## PREVIOUS ATTEMPTS AT DETECTING CYSTS IN WATER

The basic assumption in application of microscopic methods to the detection of *Giardia* cysts in water samples has been that some form of sample concentration is necessary. In 1956, Chang and Kabler(15) described a membrane filter technique for concentrating *Entamoeba histolytica* cysts in tap water. Five replicate experiments were conducted at four cyst concentrations: 1, 3, 5, and 10 cysts/3.8 liters. Each sample was membrane filtered and the retained material was washed from the membrane with about 2 ml of distilled water. This concentrate was examined microscopically in a counting chamber. Mean cyst recoveries of 20%, 28%, and 42% were reported at cyst concentrations of 3, 5, and 10/3.8 liters, respectively. No cysts were recovered from the sample containing one cyst/3.8 liters.

During the investigation of the 1965-66 Aspen, Colorado giardiasis outbreak, Moore et al(16) adapted the Chang and Kabler method to the examination of water and sewage samples for *Giardia* cysts. Two-liter water samples from 10 sites throughout the city, and one-liter sewage samples from three main trunk lines were collected. The samples were passed through cheesecloth and filtered through 0.45 μm porosity membrane filters. The sediment on the filters was brushed into water, centrifuged, and preserved in 10% formalin for microscopic examination. No cysts were detected in the 10 water samples. Two sewage samples from lines draining the eastern section of the city contained three *Giardia* cysts and one or 2 cysts of *Entamoeba coli* and *Endolimax nana*/100 ml.

In 1972, R. A. Wright (personal communication) added unknown quantities of *Giardia* cysts to one l of physiologic saline, filtered the samples through 8 μm porosity membrane filters by suction, and brushed and washed the sediment into 50 ml of saline. In 5 trials, a few cysts were invariably identified but quantitation was not attempted. Subsequently, in the investigation of an outbreak, Wright used this technique on two 20 gal stream water samples and one 5 gal sewage sample. The sewage sample was prefiltered before going through the 8μm membrane. *Giardia* cysts were not identified in these samples.

In 1973, Barbour et al(12), while investigating an outbreak in a group of campers, filtered 22 l of stream water through a membrane filter. *Giardia* cysts were not found but coccidian oocysts were observed in small numbers. With the exception of the Aspen, Colorado sewage samples, the membrane filter method has not been successful in demonstrating cysts in water samples. Whether this is a function of the inability to process a sufficient volume of water, or of the failure to adequately "clean-up" the retained material, or perhaps an indication that the organism was not present in these water samples at the time of sampling, is not known.

By the time of the Rome, New York outbreak in 1974-75, and apparently operating under the premise that larger volumes of water must be sampled to detect cysts, the CDC had developed a large-volume sampling technique(17). The method used a swimming pool filter in which sand was the filtering medium. Ten samples of raw Rome, New York water, representing a total volume of about 1.1 x $10^6$ liters (280,000 gal.) were collected by pumping water through the filter at an average flow rate of 76 liters/min (20 gpm) daily for 10 days. Filter backwash was collected each day in two 210 liter (55 gal) drums and coagulated with alum. After a 24-hour settling period, the supernatant fluid was removed and 1 to 2 liters of sediment were collected. Aliquots of each sample were fed to beagle puppies, examined microscopically at Rome, and sent to CDC for further processing.

The sediment samples examined at CDC were washed through two layers of cheesecloth and an 80 μm wire screen by gravity, and then through a 50 μm porosity membrane filter by vacuum. Most of the supernatant was removed following centrifugation at 1,000 rpm for 10 min, and selected sediment samples were examined microscopically. Two of the ten samples fed to dogs produced infection 20 and 34 days after feeding, and a cyst was observed microscopically at CDC in one of these samples. This was the first time that a cyst had been detected in a water supply, and the first time that concentrates from a water supply had been found infective for laboratory animals. The efficiency of this method has not been reported by CDC, but in one experiment where cysts were added to water at a level of 20/3.8 liters (1 gal) and 34,200 liters (9,000 gal) were sampled, no cysts were detected by microscopic examination (B. Wood, personal communication).

The CDC used this method in Camas without success but cysts were detected by the U. S. Environmental Protection Agency (EPA) in an 855 liter (225 gal) sample of distribution system water collected with the EPA method (18,19) described in a later section, and also in samples of raw surface water from the Camas supply. In simultaneous sampling by EPA and CDC in Rocky Mountain National Park in 1976(19, 20), cysts were detected in beaver pond water but only by EPA examination of the CDC concentrates. This may have reflected the difference in location of intakes for the respective samplers (the CDC intake was located on the pond bottom; the EPA intake was suspended about 0.5 m above the pond bottom), or the fact that the volume of water sampled by CDC was approximately 10 times greater than that sampled by EPA.

Another method was developed by alert laboratory personnel at the Androscoggin Valley Hospital in the Berlin, New Hampshire outbreak of 1977(3). The sampling device was a funnel consisting of a plastic bottle with the bottom cut out. A filter pad made of layers of gauze was placed in the neck of the bottle and laboratory tap water was filtered. Cysts were identified in the material trapped by the gauze but the volume of water sampled was not determined. A portion of the material retained by the filter was examined by EPA and large numbers of yeast cells were also noted.

## THE EPA METHOD

### Development

In 1976, also operating under the assumption that some form of sample concentration was necessary, EPA developed a method for detecting *Giardia* cysts in water samples. In developing a method, one would logically proceed by gathering information about the physical and chemical characteristics of the organism and pertinent facts relating to its natural history. An appropriate method would then be devised, evaluated in the laboratory, and applied in the field. In this case, pre-existing field equipment for large volume virus sampling was adapted for detecting *Giardia* cysts(18).

In February, 1976 the equipment was used for the first time in the field in Montgomery Center, Vermont to collect samples for virus analysis during an outbreak of gastroenteritis. One component of the virus apparatus is a 4 cm (10 in)-long, honeycomb yarn-wound 10 μm-porosity Orlon filter used to prevent clogging of the epoxy-fiberglass virus-adsorbing filter when sampling turbid water. Since it was known that suspected waterborne outbreaks of giardiasis had occurred previously in Vermont(21), it was decided to process the Orlon prefilter from a 1060 l (279 gal) raw stream water sample collected in Montgomery Center. The Orlon filter was backwashed with about one l of distilled water. The backwash suspension was passed through a series of nylon screens (30 to 50 $\mu$m mesh) to remove larger particulates, and the material retained by a 7$\mu$m mesh nylon screen was examined microscopically. One *Giardia* cyst was detected in this concentrate.

In May, 1976, the Camas, Washington giardiasis outbreak occurred. Using the EPA method, cysts were detected in treated distribution system water, in chlorinated influent at the filtration plant, and in raw water from 1 of the town's 2 surface supplies (1,18). In a preliminary survey of 6 sites in Washington State in June, 1976, a *Giardia* cyst was detected in treated water of the Hoquiam water supply, and a distorted cyst was detected in the raw water of the Everett supply.

By this time, the sampling device, weighing less than 3 kg (about 6 lb), consisted of a garden hose inlet, the Orlon filter holder, a plastic water meter, and a 1.9 liter/min (0.5 gpm) limiting orifice at the end of the effluent hose (Fig 1). The backwashing process was replaced with a procedure that involved cutting the Orlon fibers down to a depth of about 0.5 cm and blending the fibers at low speed in a homogenizer for 10 seconds with 250 ml of distilled water to separate the retained particulates from the filter medium. The blending procedure was adopted because, in the preliminary survey in Washington State, cysts were not detected in backwash material but were observed in the filter homogenates. It was also subsequently determined that the Orlon filter medium was superior to cellulose acetate or

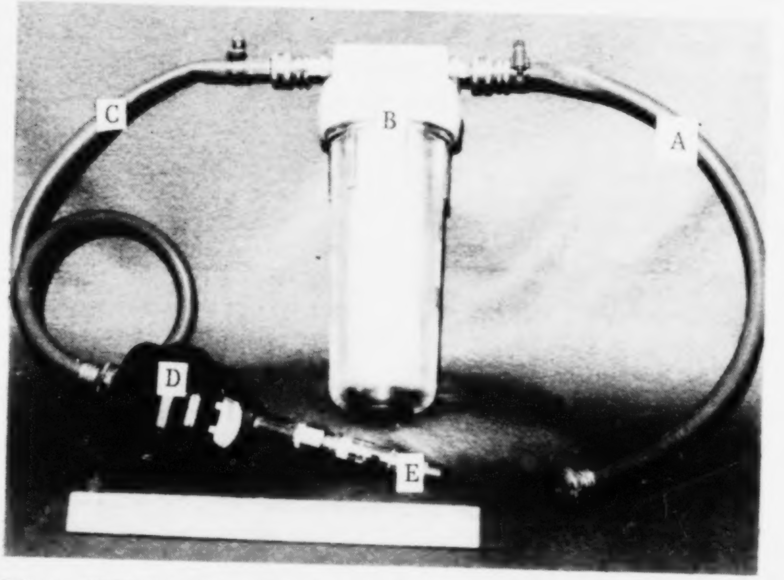

**FIG. 1.** *USEPA* **Giardia** *sampling device. A—inlet hose; B—filter housing; C—outlet hose; D—water mete; E—limiting orifice flow controller.*

polypropylene yarn-wound filters, and to epoxy-fiberglass filter tubes. The cellulose acetate and polypropylene fibers had a tendency to break upon homogenization thus contributing fibers to the concentrate that interfered with cyst detection. The epoxy-fiberglass filters also exhibited this disadvantage, and in addition, would clog during filtration at much lower volumes than would the yarn-wound filters. Modifications subsequently incorporated into the method included use of 7 μm nominal porosity Orlon filters instead of 10 μm, removal and blending of all fibers on the stainless steel filter core instead of just the upper 0.5 cm layer of fibers, and use of acid, alkaline, or acid-formalin flocculation for clarification of homogenates containing high levels of interfering particulates.

For 1977 and most of 1978, the procedure followed in examining water samples has been essentially that shown in Fig 2. Using this procedure, cysts were detected in 1977 in spring water in Granby, Colorado, in well water and surface water in Eagle County, Colorado, and in well water in Hot Springs, Colorado. Cysts were detected in the raw surface water of 2 supplies and in the distribution system water serving Berlin, New Hampshire, in additional raw stream water samples in Hoquiam, Washington, and in lake water in Conneticut. In 1978, a positive sample was obtained from stream water in Vail, Colorado.

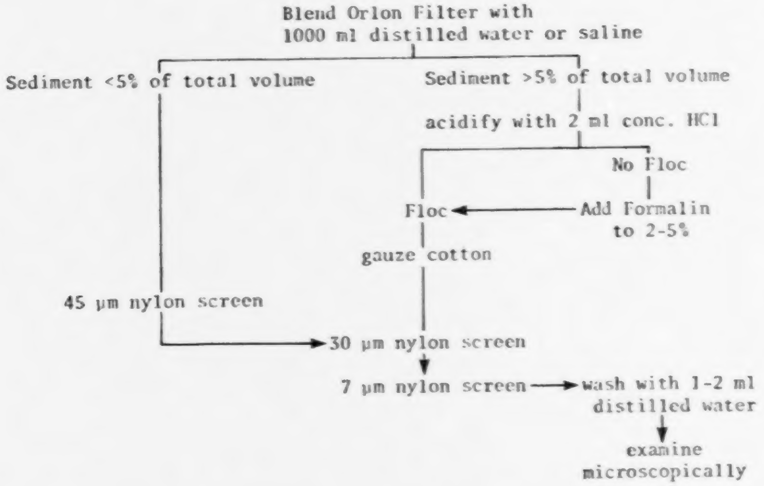
Blend Orlon Filter with
1000 ml distilled water or saline
Sediment <5% of total volume
Sediment >5% of total volume
acidify with 2 ml conc. HCl
No Floc
Floc
Add Formalin to 2-5%
gauze cotton
45 μm nylon screen
30 μm nylon screen
7 μm nylon screen
wash with 1-2 ml distilled water
examine microscopically

FIG. 2. *Processing scheme for Giardia water filters.*

## Evaluation

The EPA method had been successfully used to detect cysts in water in several outbreaks and surveys. However, a number of questions soon arose concerning the methodology: What was the efficiency of the method? What sample volume should be examined? How frequently should samples be collected? What flow rate should be used? How should samples be stored and shipped? How soon after collection must they be examined? The first problem requiring resolution was: What method should be used to count the cysts in experiments to determine efficiency of the concentrating technique?

S. L. Chang (personal communication) suggested a method in which 2 drops of cyst suspension were dispensed from a calibrated Pasteur pipet onto a slide, a coverslip was applied, and cyst counts were done of 20 random fields under low power (ca 300x). The cyst concentration was calculated by multiplying the total observed count by the number of fields available in the coverslip when using a Pasteur pipet calibrated to produce 40 drops/ml. In Dr. Chang's experience, this method was easier to use and produced more consistent results than counts obtained with a cell counting chamber. A preliminary evaluation of the coverslip and hemocytometer methods is shown in Table 1. The hemocytometer counts were obtained with an American Optical Improved Neubauer Hemocytometer. Two drops of cyst suspension were placed in the middle of the chamber, a coverslip was set in place, 20 squares were counted, and the total count was multiplied by an appropriate factor ($2 \times 10^5$) to give the number of cysts/ml.

Fifteen replicate samples of a cyst suspension were counted with both methods. The mean counts obtained with the hemocytometer were more

*Table 1. Comparison of* Giardia *cyst counts by the coverslip and hemocytometer methods*

| Subsample | Cyst Count ($\times 10^5$) | |
|---|---|---|
| | Coverslip | Cell Chamber |
| 1 | 3.12 | 6.4 |
| 2 | 3.34 | 22.4 |
| 3 | 2.79 | 44.8 |
| 4 | 2.33 | 25.6 |
| 5 | 3.22 | 38.4 |
| 6 | 1.85 | 8.0 |
| 7 | 2.42 | 25.6 |
| 8 | 2.68 | 14.4 |
| 9 | 3.01 | 12.8 |
| 10 | 2.03 | 38.4 |
| 11 | 2.22 | 22.4 |
| 12 | 1.96 | 20.8 |
| 13 | 1.71 | 19.2 |
| 14 | 1.44 | 22.4 |
| 15 | 2.15 | 20.8 |
| $\overline{X}$ | 2.42 | 22.8 |
| S.E.M.[a] | 0.15 | 2.8 |

[a]Standard error of the mean

than 9 times higher than those observed with the coverslip method and no satisfactory explanation could be found. However, the standard error of the mean for the hemocytometer method was significantly greater than that of the coverslip method even after adjusting for the higher counts observed with the hemocytometer. The variability exhibited in counts made with the hemocytometer would make interpretation of data difficult.

Modifications were made in both counting methods and another evaluation was performed. For the coverslip method, 5 $\mu$l of cyst suspension were placed on a slide, covered with a 22 x 22 mm coverslip, and sealed with a 1:1 parafin-vaseline mixture. The cyst density was calculated as follows:

$$\frac{\text{cyst no.}}{5\mu\text{l}} \times \frac{1{,}000\ \mu\text{l}}{1\ \text{ml}} = \frac{\text{cyst no. x } 200}{1\ \text{ml}} = \text{cysts/ml}$$

In the hemocytometer method, a coverslip was placed over the counting chamber and the platforms were flooded with 9 to 10 $\mu$l/platform. The 4 corner squares (1 $\text{mm}^2$ each) on each platform were counted and cyst density was calculated as follows:

$$\frac{\text{cyst no.}}{4\ \text{mm}^2} \times \frac{10}{1\ \text{mm}} \times \frac{1{,}000\ \text{mm}^3}{\text{ml}} = \text{cysts/ml}$$

Ten replicate samples were drawn from a cyst suspension and counted by both methods by each of 3 observers. The results are shown in Table 2. The mean counts with the hemocytometer were 1.7 to 1.9 times greater than those obtained with the coverslip method for the respective observers. There was no significant difference in standard error between methods after adjusting for the higher counts obtained with the hemocytometer. There was no significant difference among observers with either method except for observer No. 2 and the hemocytometer method. Observer No. 2 showed more variance with this method than with the coverslip method, recording a standard error 1.9 and 2.6 times higher than those of the other observers.

*Table 2. Evaluation of coverslip and hemocytometer counting methods for* Giardia *cysts*

| | Observer No | | |
|---|---|---|---|
| Cyst Counts | 1 | 2 | 3 |
| Cover Slip | | | |
| Mean ($\times 10^3$) | 35 | 46 | 44 |
| S.E.M.[a] ($\times 10^3$) | 1.1 | 1.5 | 1.2 |
| Range ($\times 10^3$) | 30-40 | 40-57 | 40-50 |
| Hemocytometer | | | |
| Mean ($\times 10^3$) | 68 | 86 | 73 |
| S.E.M.[a] ($\times 10^3$) | 2.2 | 5.7 | 3.0 |
| Range ($\times 10^3$) | 58-80 | 56-107 | 58-85 |

[a] Standard error of the mean

The same cyst suspension was diluted 1:400 to obtain a sufficient volume to analyze 30 replicate samples of 100 ml each on a HIAC (Pacific Scientific Co., Montclair, California 91763) particle counter. The mean number of particles counted on channels 4 thru 7 of the HIAC (representing particles 7-20 μm in size) was 91,271 or 1.2 and 2.2 times higher than the counts obtained with the hemocytometer and coverslip methods, respectively. Since the HIAC cannot discriminate between cysts and debris particles of similar cross-sectional surface area, it was not felt that this method would offer a suitable alternative to microscopic counting methods.

Based upon the above analysis and the fact that the hemocytometer requires only a small fraction of the time required with the coverslip method, the hemocytometer was selected as the method of choice for enumeration of cysts.

Late in 1977 we reported(19) on a series of 11 trials where about 1 × $10^6$ cysts were added to 380 liter (100 gal) volumes of Cincinnati drinking water which were processed for cyst recovery by the EPA method (Table 3). The mean recovery of cysts in this series was 6.3% with a range of 3 to 15%. We have since analyzed preliminary data for each step in the sample collection and processing method in order to determine where improvements may be made.

The first point at which cysts may be lost is during the collection of the sample with the 7 μm porosity Orlon filter. Experiments have not been performed using this filter to determine what fraction of *Giardia* cysts might pass through it. However, data supplied by the manufacturer(22) indicate that the honeycomb filters remove a minimum 95% of all particles at the rated porosity when processing non-aqueous fluids with a viscosity 3.5 times

*Table 3. Recovery of added* Giardia *cysts from drinking water with the EPA method*

| Trial No | Calculated Cyst Count (x$10^6$)[a] | % Recovered |
|---|---|---|
| 1 | 0.96 | 8 |
| 2 | 1.06 | 5 |
| 3 | 1.01 | 4 |
| 4 | 1.01 | 4 |
| 5 | 1.01 | 8 |
| 6 | 1.01 | 10 |
| 7 | 1.01 | 3 |
| 8 | 1.10 | 3 |
| 9 | 1.38 | 15 |
| 10 | 1.07 | 6 |
| 11 | 1.01 | 3 |
| | Mean | 6.3 |
| | S.E.M.[b] | 1.1 |

[a] Number of cysts added to 380 liters (100 gal) of drinking water
[b] Standard error of the mean

that of water at a flow rate of 11.4 liters/min(3 gpm). *Giardia* cysts are generally larger than 7 μm, and since efficiency of filtration would increase in aqueous fluids of lower viscosity and as particulates accumulated on the filter, it was assumed that retention of cysts by this filter would exceed 95%.

The next step in the process is blending the filter fibers with a diluent to recover retained particulates. As mentioned earlier, backwashing the filters had been found less successful than the blending process in finding naturally-occurring cysts in field samples. Distilled water, saline, and 1% beef extract diluents have been examined for effects on recovery by filtering cysts suspended in these media through 7 μm mesh nylon screens. No apparent advantage in terms of recovery was exhibited by any of these media (unpublished data). In the absence of data on the effects of medium tonicity on *Giardia* physiology, and since the concern was with detecting the organisms as they occur in a hypotonic medium (drinking water), distilled water was selected as the diluent.

Recovery of cysts from the Orlon filter was determined as follows: 3.8 l of drinking water containing about 1 × $10^6$ cysts (additive suspension) were continuously added to 380 l of drinking water by means of a proportioner pump while filtration progressed through the 7 μm porosity Orlon filter. A mixing chamber was present in the system between the point of cyst addition and the Orlon filter holder. The number of cysts in the 3.8 l of additive suspension was determined by filtering another 3.8 l portion of additive suspension through a 7 μm mesh nylon screen, collecting the cysts in 1 ml of distilled water and counting with the coverslip method. This count represented the challenge dose to the Orlon filter. The number of cysts recovered from the Orlon filter was determined by passing the filter homogenate through a 7 μm mesh nylon screen and performing counts in a manner similar to that for the additive counts. The percent recovery was determined by dividing the homogenate count by the additive suspension count and multiplying by 100. The results are shown in Table 4.

A mean recovery of 58% of cysts was obtained from the Orlon filter with a range of recovery of 12 to 139%. Assuming that only an insignificant percentage of cysts would pass through the filter (based on manufacturer's data) the relatively low and highly variable recovery may be due to difficulties in preparation of the homogenate. The homogenate is obtained by blending 1/4 of the Orlon filter at a time with 250 ml of distilled water at low speed (17,000 rpm) for 10 seconds in a 473 ml (1 pint) blender jar. It is extremely difficult to perform this procedure in exactly the same fashion from sample to sample. The blender blades will often become entangled in the Orlon fibers thus reducing the blending speed and effectiveness of the process. Cysts may also be lost while separating the particle suspension from the Orlon. In this procedure, the aqueous portion is separated from the fiber mat by decantation after blending. The fiber mat is then placed into a plastic bag with one corner removed and the remaining fluid is expressed from the fibers. Cysts would undoubtedly adhere to or become entrapped in the filter fibers at this point.

*Table 4. Cyst recovery from Orlon filter homogenate*

| Trial No. | | % Recovered |
|---|---|---|
| 1 | | 12 |
| 2 | | 26 |
| 3 | | 18 |
| 4 | | 41 |
| 5 | | 99 |
| 6 | | 77 |
| 7 | | 28 |
| 8 | | 48 |
| 9 | | 139 |
| 10 | | 79 |
| 11 | | 66 |
| | Mean | 58 |
| | S.E.M.[a] | 12 |

[a] Standard error of the mean

In a filter homogenate not requiring flocculation (one with <5% sediment), the next step in the concentration process that has been evaluated is the recovery of cysts on the 7 μm nylon screen. Three trials were conducted in which Orlon filters were challenged with about $1 \times 10^6$ cysts using the proportioner method described earlier and 380 liter volumes of drinking water. Aliquots of the filter homogenate were concentrated with a 7 μm mesh nylon screen and the cysts were collected in 1 ml of distilled water, or by centrifuging the homogenate at 1,500 rpm for 10 min, suspending the sediment in 1 ml of the supernatant fluid, and counting the cysts. The results are presented in Table 5. Mean recovery with the nylon screen was 7.8%, and with the centrifugation procedure, 32%. These results indicated that about 71% of the cysts in the homogenate were not being recovered with the nylon screen procedure when compared to recovery by centrifugation.

Recovery of cysts with the nylon screen was investigated further in another series of experiments in which polycarbonate (PC) membrane filters and centrifugation were also compared. Replicate 50 ml samples containing about $1 \times 10^6$ cysts in water were vacuum filtered through 47 mm diameter 7 μm mesh nylon screens or 8 μm porosity PC membrane filters (Bio-Rad, Richmond, California 94804) and retained cysts were collected in 1 ml of

*Table 5. Cyst recovery from Orlon homogenate by nylon screen vs centrifugation*

| | % Recovery | | % Loss |
|---|---|---|---|
| | 7 μm Nylon Screen | Centrifugation | with Screen |
| | 2.7 | 40 | 93 |
| | 14.6 | 24 | 40 |
| | 6.1 | 32 | 81 |
| Mean | 7.8 | 32 | 71 |

water. Filtrates from the screens and PC filters were centrifuged at 1,500 rpm for 15 min, and the sediment was resuspended in 0.5 ml of supernatant fluid. Replicate 50 ml samples of cyst suspension were centrifuged without filtration and concentrates were prepared in the same manner as for the filtrates. All counts were performed with a hemocytometer. Percent recovery was calculated by dividing the cyst counts obtained in the concentrates by the cyst count of the original suspension and multiplying by 100. Results are shown in Table 6.

The mean recoveries for 5 trials were 46%, 64%, and 132% for the screen, PC filters and centrifugation, respectively. Centrifugation analysis of the filtrates indicated that 16% of the cysts were passing through the screens and 0.9% through the PC filters. Adding the surface recoveries to those obtained in the filtrates results in overall recoveries of 62% for the screens and about 65% for the PC filters. The loss of 35 to 38% of the cysts may have been due to adsorption of cysts to screen and filter material with subsequent failure to recover these with the small volume (1 ml) of wash water used. The >100% recoveries by centrifugation may have been due to counting error from the heavy cyst suspension in the final concentrate (>1 × $10^6$/0.5 ml).

*Table 6. Comparison of nylon screen, polycarbonate filter, and centrifugation for recovery of* Giardia *cysts*

| | % Recovery | | |
|---|---|---|---|
| Trial No. | 7 µm Nylon Screen | 8 µm PC Filter | 1500 rpm, 15 min Centrifugation |
| 1 | 46 | 65 | 118 |
| 2 | 44 | 49 | 149 |
| 3 | 43 | 75 | 122 |
| 4 | 41 | 69 | 133 |
| 5 | 55 | 62 | 140 |
| $\overline{X}$ | 46 | 64 | 132 |
| S.E.M.[a] | 2.5 | 4.3 | 5.7 |
| | Screen Filtrate | PC Filtrate | |
| 1 | 12 | 2 | |
| 2 | 15 | 0.7 | |
| 3 | 13 | 1.1 | |
| 4 | 19 | 0.2 | |
| 5 | 20 | 0.5 | |
| $\overline{X}$ | 16 | 0.9 | |
| S.E.M.[a] | 1.6 | 0.3 | |

[a] Standard error of the mean

The experiments described in the above sections were performed with cysts suspended in clear waters relatively free from other particulate matter. Many waters contain significant quantities of suspended particulates that are retained by the Orlon filter and released into the homogenate thus complicating detection of cysts. In the processing scheme shown in Fig. 2, the presence of >5% sediment in the filter homogenate was arbitrarily selected as the level at which clarification procedures (flocculation, gauze and nylon screen straining) would be used. Evaluation of recovery efficiency when these technics are employed has not been performed. Of the clarification steps listed in Fig. 2, flocculation would be the one most likely to result in significant cyst loss.

In trial no. 1 listed in Table 3, one aliquot of the homogenate was processed only with the 7μm mesh nylon screen. Another aliquot was subjected to acid flocculation and strained through gauze and 45 and 30 μm mesh nylon screens before collection on the 7 μm mesh screen. The recovery of cysts was 0.6% when flocculation was used as compared to 8% with only the 7 μm mesh screen, representing more than a one logarithm loss of cysts by flocculation and preliminary straining.

## Suggested Modifications To The EPA Method

The 7 μm porosity Orlon filter is probably more than 95% efficient in retaining *Giardia* cysts in water samples. The blending technique for cyst recovery from the filter is inadequate for high-efficiency reproducible cyst recovery. The availability of a backwashable filter medium that could process large volumes of water would greatly enhance a detection method for *Giardia*. Preliminary contacts with filter manufacturers indicate that a need for a backwashable filter does exist, at least in the pharmaceutical industry, and developmental research is underway.

The use of the 7μm mesh nylon screen as the final step in collection of cysts (Fig 2) is no longer recommended. Results presented in Tables 5 and 6 indicate 58 to 71% loss of cysts with these screens. A portion of the lost cysts pass through the screens and others become enmeshed in, or adsorbed to, the screen material. Using 8 μm porosity PC filters results in an increase of about 50% in recovery over the nylon screen (Table 6), and centrifugation produced a 4-fold increase in recovery as compared to the nylon screen (Table 5). Recent experiments in our laboratory (unpublished data) indicated up to 81% recovery of cysts on 5 μm porosity PC filters. Therefore, it is recommended that the final concentration process substitute either centrifugation (1,500 rpm, 15 min) or membrane filtration (5 μm or less porosity) for 7 μm mesh nylon screen filtration.

The use of flocculation on a given sample must weigh decreased cyst recovery against a possible increase in masking effects caused by the presence of particulate matter. This decision should be based upon the experience of the investigator and the nature and quantity of the particulate matter. Homogenates from a single sample may be divided and processed by different techniques to determine which results in optimum recovery. The use of flotation techniques may be a satisfactory alternative to flocculation as a means for sample purification but these techniques have not been evaluated in our laboratory. Inorganic particulates, such as clays, should not present a problem in separation because of their higher density. Flotation may not prove suitable for cyst purification if organic particulates with densities similar to *Giardia* cysts are present.

## SAMPLING CONSIDERATIONS

### Volume, Frequency, And Flow Rate

Questions of sample volume, frequency, and flow rate are not peculiar to *Giardia* detection. These questions exist with regard to other microbiological and inorganic contaminants of water supplies as well. Their resolution is usually dependent upon the purpose of the survey, the efficiency of the method, and economic and practical factors rather than consideration of statistically sound sampling guidelines. In 1977, a sample volume of 2,000 l (500 gal) was suggested for surveys on the occurrence of *Giardia* cysts in water supplies (19). This suggestion was based on the assumption that it would be desirable to detect *Giardia* at a level of 1 cyst/20 l and that the efficiency of the method was 1%. It is our opinion now that the sample volume should be reduced to 380 l (100 gal) if the centrifugation or membrane filtration process is substituted for the 7 μm mesh nylon screening, especially if turbid waters are being sampled (i.e., those resulting in >5% sediment in the filter homogenate). There is no advantage in collecting a 2,000 l sample over a 380 l sample if sensitivity in the larger sample volume is decreased one logarithm by the need for flocculation or other clarification techniques.

Flow rates of 11.4 liters/min (3 gpm) at line pressures of 15 to 70 psig have usually been used in *Giardia* sample collection. No evaluation of flow rate on recovery of cysts has been performed. In outbreak investigations, attempts are made to sample historic water within the distribution system. Conditions at the outbreak site will usually dictate sample volumes collected and flow rates used. There is currently no basis on which to establish frequency of sampling recommendations.

## STORAGE, SHIPMENT, AND PROCESSING

There are no reliable data on the effects of different storage temperatures on *Giardia* cysts. A study performed in our laboratory on cysts stored at −70, −20, 5, 20, and 37°C indicated no appreciable change in cyst counts over a 2 week period with the exception of those stored at −20°C (unpublished data). Cyst counts decreased by about 40% after one week storage at −20°C; possibly due to aggregation of the cysts. No attempt was made to determine viability or infectivity of stored cysts. It would seem prudent to store and ship filter samples at refrigerator temperatures (ca 5°C) and to process samples as soon as possible after collection. Higher temperatures could result in growth and activity of other organisms that might be detrimental to cyst recovery.

Cyst concentrations in water samples may be estimated by multiplying the number of cysts observed in a measured volume of concentrate by the appropriate conversion factor for the counting chamber used and the reciprocal of the fraction of concentrate examined. In our laboratory, a sample is considered negative when no cysts are detected in four 5 μl aliquots of the 1 ml sample concentrate.

## SIGNIFICANCE OF RESULTS

Not much can be said concerning the safety of a supply if a cyst is detected in a water sample. At the present time, there is no easy method for determining the host species of origin of the cyst. Since *Giardia* attacks a wide variety of animal species and little is known of the host range of *Giardia* species, the interpretation of a positive finding is open to question. In other words, a detected cyst may be host specific for a species other than man. Furthermore, microscopic examination alone yields no information on the viability or infectivity of detected cysts. Infectivity of cysts can be determined only by animal feeding experiments. The recent development of an excystation technique(6) may provide a means for determining viability. However, the relationship of viability by excystation to infectivity remains to be determined.

At the present time, it is suggested that the use of the concentration method for detection of cysts in water samples be limited to outbreak investigations or to examination of water supplies where concurrent surveys of watershed animals and/or epidemiologic studies are being conducted. The efficacy of treatment techniques for removal of cysts can be determined only if raw and treated water samples are examined. A negative sample does not necessarily mean that a water supply is safe since this finding may reflect intermittent contamination of the supply, poor recovery efficiency with a given water, or insufficient sample volume or sampling frequency.

## SUMMARY

The development and application of methods for detection of *Giardia* cysts in water samples have been reviewed. Microscopic detection methods appear to be the only presently available practical approach for conducting occurrence surveys in water supplies. The level of cysts in raw sewage was estimated to be between $9.6 \times 10^3$ to $2.4 \times 10^5$ cysts/liter when 1 to 25% of the population is infected.

Evaluation and application of a large volume sampling and processing method developed by EPA has been described. A step-by-step analysis of the procedure indicates a cyst loss of 42% to the Orlon filter in the preparation of the homogenate, 58 to 71% loss in the 7 μm mesh nylon screening process, and more than 90% loss in the flocculation step. Suggested modifications to the method that may result in a four-fold increase in efficiency of recovery (substitution of centrifugation or membrane filtration for the 7 μm mesh nylon screen) were described and evaluated. Other modifications that remain to be developed and investigated include the use of backwashable filters and the substitution of flotation techniques for flocculation as a clarification procedure.

A sample volume of 380 liters (100 gal) is suggested for occurrence surveys in water supplies with concurrent epidemiologic and/or watershed animal

surveys. Interpretation of positive findings is dependent upon development of methods for differentiating *Giardia* species derived from a variety of animal hosts, ascertaining the host range of the organisms, and determining the infectivity of detected cysts.

## ACKNOWLEDGMENT

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## Discussion

T. NASH: Taking the recoveries that you had in the techniques that were used, can you calculate numbers of cysts you were finding per gallon in the epidemic study?

W. JAKUBOWSKI: That is really impossible to say at this point. Initially we were pleased just to be able to detect cysts. In many cases, this was after examination of several cover slip preparations. Perhaps a volume in the range of 20 microliters out of 1 milliliter of concentrate may have been examined microscopically. Generally, when a cyst was detected in a sample we did not look any further. We usually considered the sampling negative after an examination of four 5-microliter portions yielded no cysts.

Initially, we were not trying to calculate levels of cysts. One of the highest that we did detect was about 50 cysts in 225 gallons of distribution system water. We did do some extrapolation to reach this figure — although we cannot depend upon this extrapolation, since we did not have any information on the efficiency of the method used in that particular water.

A. TOMBES: Your schematic showed that you washed off your 7 $\mu$m screen. Would you then withdraw 1 or 2 samples of 5 $\mu$l volumes to examine for determining cyst concentration?

W. JAKUBOWSKI: Our guideline is that a sample is negative when four 5 $\mu$l portions have been examined without detecting cysts. In positive samples, further examination of concentrate aliquots were not performed when cysts were detected. In other words, if the first 5 $\mu$l portion was negative and the second 5 $\mu$l portion contained 1 cyst, the level in the concentrate would be estimated as 1 cyst/10 $\mu$l.

# The Propagation of *Giardia* Trophozoites In Vitro

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## ABSTRACT

*Giardia* trophozoites have proven difficult to propagate in vitro. Of the three morphologic types *(G. duodenalis, G. muris, G. agilis),* only organisms of the *G. duodenalis* type, of which *Giardia* from humans is an example, have been cultured. Although initially *Giardia* were isolated and cultured in the presence of yeast, then separated from it, viable yeast is not required for *Giardia* propagation. Cultures of these organisms have been initiated in the absence of any other symbiont. All of the culture media presently employed for *Giardia* propagation are complex and incorporate some type of animal serum. A brief account is presented of the methods used to isolate and propagate trophozoites of the *G. duodeoalis* type.

For the purpose of studying *Giardia* isolated from water supplies it would be ideal to be able to routinely induce excystation, then to establish and study the resultant trophozoites in culture. Although *Giardia* excystation, with the emergence of motile trophozoites, is now a process that can be regularly induced in the laboratory, the establishment of these organisms in culture remains difficult. Most of the *Giardia* cultures now available were started from trophozoites obtained from human or animal small intestine.

Of all of the common intestinal protozoa, organisms in the genus *Giardia* have proven among the most difficult to establish in culture. Even now, only a relatively few of these organisms have been isolated, and difficulties with their maintenance have resulted in their study in relatively few laboratories.

Of the three recognized morphologic types of *Giardia*, named *G. agilis, G. duodenalis*, and *G. muris* by Filice(1), only isolates of the *G. duodenalis* type have been successfully cultured in vitro. Apparently there are no reports of the attempted culture of *G. agilis*. Several workers have reported unsuccessful attempts to culture *G. muris* (2-4). The failure of *G. muris* to grow in media that support *G. duodenalis* suggests that, despite their morphologic resemblance, *G. duodenalis* and *G. muris* have significant physiologic differences.

Reports of in vitro maintenance and culture of *Giardia* trophozoites began appearing more than a half century ago. Within a few years of each other, three workers, Chatterjee(5) in 1927, Penso(6) in 1929, and Poindexter(7) in 1931 independently reported keeping *Giardia* trophozoites alive in vitro for up to 5 weeks.

In 1960, Iwata and Araki(8) reported the results of their efforts to culture *Giardia* trophozoites from humans in vitro. They used a variety of complex media, all of which contained pig liver infusion, rabbit serum and antibiotics. The maximum period of survival they observed was 12 days.

Trophozoite multiplication was observed in all cases on the second culture day and in some cases on the third or fourth days. In the most favorable medium, trophozoites survived for 12 days. They believed that bacterial overgrowth was responsible for termination of *Giardia* growth.

Karapetyan's work marks the breakthrough in *Giardia* cultivation. In 1960(9), he reported the method he had used to culture *Giardia* trophozoites from humans in vitro for 7 months. He obtained trophozoites together with *Candida guillermondii*, by duodenal intubation, concentrated them by centrifugation, and inoculated the mixture into bottles previously seeded with chick fibroblasts. His complex culture medium included serum (human, horse or beef serum was successful), chick embryo extract, chick amniotic fluid or a tryptic digest of meat, and Hanks or Earl's solution. The culture medium was changed daily. During the first few weeks the fibroblasts were gradually destroyed; thereafter the *Giardia* and *Candida* multiplied, apparently symbiotically. In a modified system, Karapetyan cultured *Giardia* from the rabbit and from man for up to 5 months in a modified system in which *Saccharomyces cerevisiae* was used instead of *Candida* and fibroblasts were not employed(10). Karapetyan reported unsuccessful attempts to axenically culture *Giardia* trophozoites. The failure of *Giardia* to grow without yeast led Karapetyan to believe that there may be some synergistic relationship between these organisms.

A number of workers later reported success with Karapetyan's method or with modifications of it. Solov'ev(11)in 1971 studied the effect of varying the serum concentration in Karapetyan's medium on the organism's growth response. He noted that the serum concentration could be reduced from 25 to 10 percent without significantly impairing the growth response. Several French workers also reported successful *Giardia* culture using Karapetyan's methods. Roux and Ecalle in 1968(12) and Ecalle(13) in a separate paper that same year studied the effect of whole pancreatic juice and of three pancreatic enzymes on the in vitro growth response of *Giardia* from the rabbit. In 1971, Gayrel and Ecalle(14) reported that changing the interval of *Giardia* medium renewal changed the time of appearance of the organism's exponential growth phase.

During the late 1950s, we in Oregon also were attempting to culture *Giardia* trophozoites. We had not found a successful medium when Karapetyan's papers became available. We immediately attempted to duplicate Karapetyan's work but were unsuccessful. In retrospect we identified our problem. We were using Karapetyan's medium, but we were not using Karapetyan's organism. We were trying to culture *G. muris* because it was readily available, not suspecting that this organism would behave differently from *G. duodenalis* organisms from the human or rabbit. We finally attempted to culture the rabbit organisms using Karapetyan's medium, and the growth response was so striking that it was clear to us that Karapetyan was onto something.Subsequently we found that by modifying Karapetyan's method we could culture *Giardia* from the rabbit and chinchilla(15) monaxenically with *S. cerevisiae*.

Axenic cultures of *Giardia* were only obtained in 1970(16). We approached this problem by assuming that the presence of living yeast was not essential, and that the contribution of the yeast might be either to provide nutrients or, as a result of its growth, to lower the redox potential. We induced the *Giardia* and yeast to grow in a yeast extract-containing medium with a low redox potential, then attempted separation of the *Giardia*.

We finally obtained axenic *Giardia* cultures by means of a U-tube (Fig. 1). *Giardia* and yeast were inoculated in the right arm of the tube. The yeast, being nonmotile, settled to the base of the right arm of the tube and remained there. The *Giardia* trophozoites, being motile, migrated across the tube. The tube could be viewed from below with an inverted microscope and the progress of the trophozoites across the tube could be determined. After about a month *Giardia* could be seen at the base of the left arm of the tube free of yeast. These *Giardia*, now free of yeast, were inoculated into tubes of the same culture medium, where they failed to grow.

These organisms would grow across a dialysis membrane from viable *S. cerevisiae* (Fig. 2). Periodically we attempted to subculture these *Giardia* axenically, and after a month of trying, axenic cultures of trophozoites from the rabbit, chinchilla and cat were obtained(16). Subsequently we succeeded, using similar methods, in isolating and culturing *Giardia* from humans(17) and guinea pigs(18).

Insert Fig. 2

The separation of *Giardia* from yeast is probably the most tedious part of obtaining an axenic culture of *Giardia*. We know now that there are easier ways of separating these organisms. One is the judicious use of an antifungal

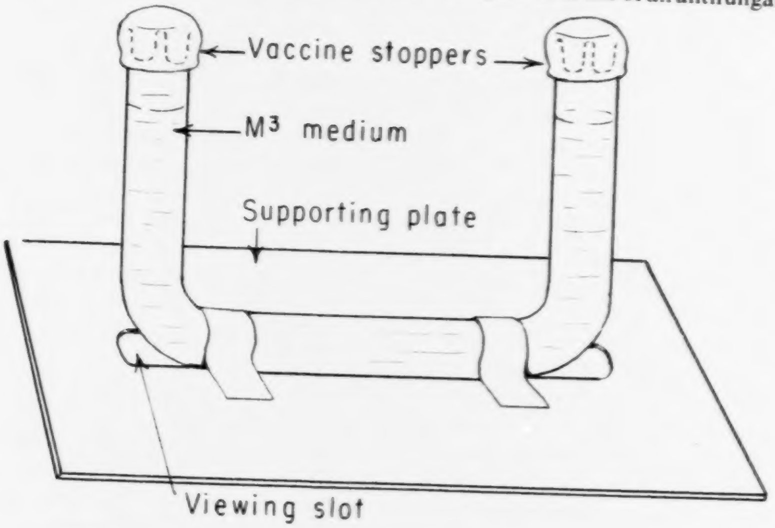

**FIG. 1.** *U-tube used to obtain* Giardia *trophozoites free of* S. cerevisiae.

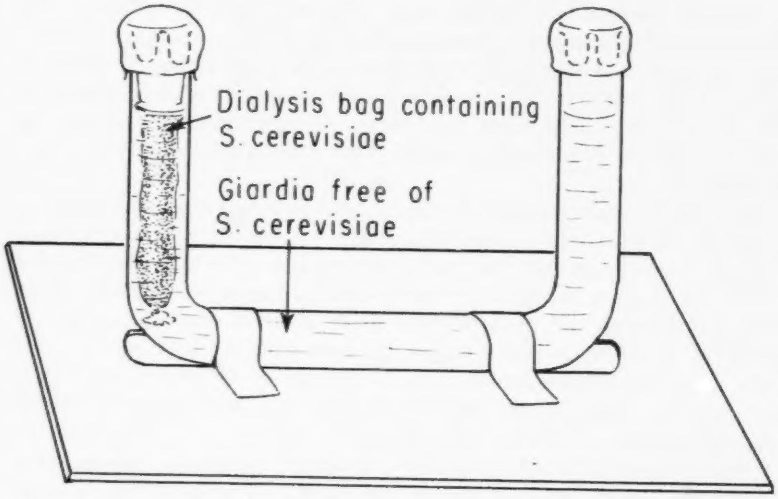

**FIG. 2.** *U-tube in which* **Giardia** *trophozoites are cultured across a dialysis membrane from* **S. cerevisiae.**

agent, slowly increasing the amount added to the culture until only a small amount of *Saccharomyces* remains, at which time subcultures are often free of yeast.

Another way of obtaining axenic *Giardia* cultures is to start without yeast. Bingham (See Bingham, these *Proceedings*) has shown that it is possible to initiate axenic cultures from treated cysts, first concentrated and purified from human or monkey feces.

Other workers are culturing and studying these organisms. Visvesvara et al(19) succeeded in adapting *Giardia* from humans to grow in media containing either bovine or rabbit serum. This is an important contribution because organisms grown in medium containing human serum are not suitable for use in the indirect immunofluorescence test for the detection of antibodies to *Giardia* in the serum of patients.

Finally, Lakhonina in Estonia(20) has reported axenically culturing 13 strains of *Giardia* from the rabbit; she and Teras(21) have reported that adding 1 to 1.5% agar to *Giardia* media offers several advantages. It permits slower growth of the organisms and medium changes at less frequent intervals and it is a medium in which the ability of *Giardia* to produce acid or gas from a variety of sugars can be tested.

Presently, then, methods are available to isolate and culture organisms of the *G. duodenalis* type. The methods are difficult and we are working to develop simplified procedures that will permit the widespread study of these organisms. *Giardia* have not yet been cultured on a defined medium and this is another objective to which we are directing our attention. Organisms of the *G. muris* type remain a challenge to culture; no one up to now has apparently even come close. And *G. agilis*, the representative of this genus found in coldblooded vertebrates, remains largely unknown; a search of the literature yields no reports of its attempted culture.

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## Discussion

M. WOLFE: In the relationship of yeast to trophozoite growth, if yeast flora were present in the small bowel, it might help to support new growth of *Giardia* and perhaps work synergistically towards some pathogenic effect. Although I do not know what factors would induce yeast growth in human beings, I believe this is a possibility that gastroenterologists should consider.

Can you give us some idea of the effect of the presence, or absence, of yeast in the fluid of the small bowel?

E. MEYER: It would not surprise me if yeast is present in the small bowel and aids in the growth of *Giardia*. However, I believe that its presence is not essential for *Giardia* growth in the small intestine. Bemrick (Bemrick, W. J. and M. K. Grady, J. Parasitol. 51:685, 1965) wondered about the need for yeast in the small intestine. As a result, he examined a large number of sacrificed dogs to determine what per cent contained *Giardia* alone in the small intestine, yeast alone, or *Giardia* plus yeast. His conclusion was that there is no relationship to incidence of *Giardia* and yeast in the small intestine although you often find the 2 together.

D. JURANEK: Is there a morphological difference between *G. agilis* and *G. muris* that is obvious?

E. MEYER: *G. agilis* are much longer and much thinner. They have teardrop shaped median bodies. *G. muris* and *G. duodenalis* in a stain, look similar except for the median bodies, while the *G. agilis* is perhaps twice as long and considerably narrower.

L. DIAMOND: In reference to the comment Dr. Wolfe made about the relationship between yeast and *Giardia*, I would suspect there is no relationship of the type under discussion.

Have you varied the amount of serum that you have used in attempts to culture various types of *Giardia*? How many strains from humans have you been able to isolate?

E. MEYER: I do not know if you can consider them strains or not. We have cultured them from 2 or 3 different people. For the culture of trophozoites, one of my students determined the requirement for serum. He went from 0 to 95 per cent serum. Below 15 to 20 per cent, you encounter more difficulties; however, there are reports that you can successfully use 10% serum. That has not been our experience.

L. DIAMOND: With *histolytica* there are some strains which require 15 to 30% serum for axenization. Once they have been axenized, then we can drop down to the usual 10 per cent. We have cultured some at 5 per cent, but I have never been able to axenize any at 5 per cent serum.

E. MEYER: If you are trying to axenize, it would be a good idea to vary the serum concentration.

# Induction of *Giardia* Excystation And The Effect of Temperature on Cyst Viability as Compared by Eosin-Exclusion and In Vitro Excystation*

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## ABSTRACT

Factors involved in *Giardia* excystation have been investigated. *Giardia* excystation has been shown to be a pH-dependent process unaffected by the presence of gastric salts or pepsin. A method for the in vitro excystation of *Giardia* has been developed and utilized to induce excystation in cysts obtained from humans, monkeys, dogs, rats and mice.

The viability of *Giardia* cyst suspensions was compared by the excystation procedure and eosin-exclusion. Eosin-exclusion consistently indicated higher cyst viability than could be demonstrated by in vitro excystation%

Using excystation as the criterion of viability, the effect of four storage temperatures (-13, 8, 21 and 37°C) on *Giardia* survival in water was studied.

Storage at 8°C permitted longest cyst survival — upwards of 77 days. Cysts stored at 21°C retained their viability for 5 to 24 days, while those at 37°C never survived longer than 4 days. Freezing and thawing of *Giardia* cysts resulted in an almost complete loss of viability although a low level of viability (less than 1%) persisted for at least 14 days.

## EXCYSTATION OF *GIARDIA*

The information which is available describing excystation of intestinal protozoans is largely based on in vivo and in vitro research on *Entamoeba*, *Eimeria*, and *Giardia (1)*. Of these genera, the latter has been studied least.

Many of the early descriptions of *Giardia* excystation were based on in vivo observations. In 1925, Hegner (2) described *Giardia* excystation after having observed the process in a patient's stool. Later, he reported observing the process in the intestine of rats 20 min to 4½ h following peroral administration of *Giardia lamblia* cysts (3). From this experiment, Hegner concluded that moisture, temperature, and certain unspecified digestive juices were major factors in the excystation process. In 1937, Armaghan (4) attempted to pinpoint the site of *Giardia* excystation by placing suspensions of *Giardia muris* cysts directly into the stomach, duodenum, jejunum, ileum, and caecum of several rats. After 8 days, she found that only those rats

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which had cysts placed in the stomach or duodenum were excreting cysts, while none of the other rats was found to be infected at autopsy 18 days later.

Deschiens (5) found dead trophozoites in gastric juice and aqueous hydrochloric acid to which he had exposed *Giardia* cysts during an attempt to determine if cysts survived gastric passage. This was apparently the first in vitro excystation of *Giardia*. However, it was not until the work of Bingham and Meyer (6) that a factor inducing *Giardia* excystation was demonstrated. Cysts were exposed to solutions of varying pH, ranging in complexity from complete synthetic gastric juice to aqueous hydrochloric acid. This experiment, which is summarized in Table 1, showed that the hydrogen ion, chloride ion, or a combination of the two was responsible for inducing excystation.

*Table 1. Percentage excystation of* Giardia *cysts exposed to complete and component-varied synthetic gastric juice.*

| Solution | pH | Mean* % excystation ± S.E.M. |
|---|---|---|
| Complete (HCl + salts + pepsin) | 1.6 | 25.8 ± 7.3 |
| HCl + salts | 1.6 | 23.0 ± 7.0 |
| HCl only | 1.6 | 23.2 ± 7.0 |
| Water (control) | 6.8 | 0.1 ± 0.1 |
| HSP-3 (control) | 6.8 | 0.0 ± 0.0 |

* Means are derived from a total of 1191 to 1724 cysts which were counted in each solution.

A subsequent experiment using a variety of inorganic acids (Table 2) confirmed that the hydrogen ion was required to initiate excystation regardless of the counter ion used. These results are similar to findings reported for other cyst-forming protozoans. For example, Kaushal and Shukla (7) found that treatment of cysts with HCl and trypsin accelerated excystation of *Hartmannella culbertsoni*.

More recently, unpublished data from our laboratory indicate that additional factors are involved in excystation. Table 3 shows the excystation response of cysts exposed from 5 minutes to 4 hours to aqueous solutions of HCl at various pH values. The following were noted. First, lowering the pH

*Table 2. Percentage excystation of* Giardia *cysts exposed to inorganic acids at pH 2.0 to 2.1.*

| Acid | Mean* % excystation ± S.E.M. |
|---|---|
| Water (control) | 0.2 ± 0.2 |
| HCl | 17.4 ± 2.2 |
| $HNO_3$ | 12.5 ± 3.0 |
| $HClO_4$ | 15.5 ± 2.0 |
| $H_2SO_4$ | 15.5 ± 2.6 |
| $H_3PO_4$ | 19.4 ± 2.8 |

* Means are derived from a total of 2096 to 2392 cysts which were counted in each solution.

decreases the exposure time required for excystation. Second, it appears that for acid pH values there exists an optimum range of exposure times above and below which significantly lower levels of excystation occur. Third, the mean optimum exposure time within this range appears to increase as the pH increases. Additionally, the mean optimum percentage of excystation is greater at low pH (0.5 to 2.0) than at higher ones. The maximum level of excystation at pH 0.5 and 2.0, while not significantly different from each other, are significantly greater than those at pH 4.0 and 6.2 ($p<0.01$). The higher mean optimum excystation percentage at pH 0.5 and 2.0 indicate that the excystation process 1) is favored by low pH values and 2) has adapted to proceed optimally at pH values which correspond to those occurring normally in the human stomach.

The medium into which cysts are transferred after exposure to acid is also critical to the excystation process. It can be seen from Table 4 that transfer of acid-exposed cysts to either water or HCl yields negligible excystation. On the other hand, transferring such cysts to HSP 3 medium (8) routinely used to culture *Giardia* trophozoites results in significant levels of excystation. This suggests that while the hydrogen ion activates the excystation process, subsequent transfer to a favorable medium is required for the completion of the process. HSP-3, the only medium that has been tested in this system, appears to be favorable but it is possible that other physiological solutions may also prove satisfactory. Similar observations have been made with other protozoans. For example, Yorke and Adams (9) observed that suspending *Entamoeba histolytica* cysts in water at 37°C was not deleterious to the cysts, but was lethal to excysting amoebae. These amoebae were often destroyed by the water before they could escape their envelopes.

Temperature has also been identified as important in the excystation process. The temperature of both the acid solution and the subsequent excystation medium (HSP-3) appears to be crucial. The results presented in Table 5 demonstrate that high levels of excystation will occur when the temperature of both is 37°C. Lower levels of excystation occurred when certain temperature combinations were used, but no excystation was observed when the temperature of the HSP-3 was held at 8°C regardless of the temperature of the HCl.

The process of *Giardia* excystation appears to be similar to that of excystation of metacercariae of the trematode, *Paragonimus westermani*, which also excyst in host small intestine. Metacercariae of *P. westermani* excysted when exposed to a medium consisting of an isotonic salt solution at 40°C and pH 9, following initial exposure to an acid medium of pH 3 (10).

## EFFECT OF TEMPERATURE ON CYSTS

With in vitro excystation of *Giardia* as an established procedure, it is now possible to re-assess earlier reports on other cyst characteristics such as thermal sensitivity. Using eosin-exclusion as their criterion for viability, Boeck (11) reported a thermal-death point of 64°C for *G. lamblia* cysts, while Cerva (12) reported 50°C as the upper limit of temperature tolerance.

*Table 3.* Giardia *excystation with pH of acid solution and time of exposure varied.*

| pH | Exposure time (minutes) | | | | | | | | | | Mean optimum exposure time+ | Mean optimum % excystation++ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 60 | 90 | 120 | 180 | 240 | | |
| 0.5 | 243* | 256 | 231 | --** | 127 | 38 | -- | -- | -- | -- | 10 | 243 |
| 2.0 | 28 | 248 | -- | 222 | 203 | 100 | 53 | 20 | -- | -- | 20 | 224 |
| 4.0 | 0 | 1 | -- | 5 | 28 | 23 | 2 | 9 | 2 | -- | 45 | 26 |
| 6.2 | -- | -- | -- | -- | 0 | 3 | 8 | 6 | 4 | 0 | 120 | 5 |

* Values represent percentages of excystation with reference to control tube (pH 2.0, 60 minutes) which is arbitrarily 100%. Underlined values represent optimum percentages of excystation at a given pH which are not significantly different from each other (p>0.01).

** Excystation was not determined at these combinations.

+ Average of exposure times at which optimum percentages of excystation were not significantly different from each other at a given pH.

++ Average of optimum percentages of excystation which are not significantly different from each other at a given pH.

*Table 4. Percentage excystation (± S.E.M.) of* Giardia *cysts exposed to various HCl solutions then transferred to either HCl solutions at the same pH as the original solution, water at pH 6.8, or HSP-3 at pH 6.8.*

| | Incubation media | | |
|---|---|---|---|
| pH | Water | HCl | HSP-3 |
| 0.5 | 0.1 ± 0.2 | 0.1 ± 0.2 | 2.0 ± 0.8 |
| 2.0 | 0.1 ± 0.2 | 0.1 ± 0.2 | 15.4 ± 2.7 |
| 4.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 17.3 ± 2.3 |
| 6.2 | 0.1 ± 0.2 | 0.0 ± 0.0 | 0.3 ± 0.3 |

Additionally, Boeck (13), using the same criterion of viability, noted that at temperatures from 12 to 22° C *Giardia* cysts remained viable for as long as 66 days. However, the eosin-exclusion method of determining cyst viability has been criticized as unreliable for determining the viability of *Entamoeba histolytica* cysts (14,15). Earlier workers preferred in vitro excystation as the criterion of *E. histolytica* cyst viability.

In our laboratory, we have compared eosin-exclusion to in vitro excystation as methods for evaluating *Giardia* cyst viability. Cysts were purified by a modification of other procedures (16,17) as follows: Feces are emulsified to a thin consistency in tap water, filtered through a layer of cheese cloth to remove large particles, and 3 to 5 ml of filtrate overlaid on 3 ml of chilled 0.85M sucrose in a 15 ml conical centrifuge tube. The tube is centrifuged at 600g for 5 min at room temperature and the water-sucrose interface removed, diluted 1:10 with tap water, and recentrifuged for 5 min. The pellet is resuspended in 3 ml of water and the sucrose gradient centrifugation repeated. Following this the water-sucrose interface is again removed, diluted 1:10 with water, filtered through a 20' m-aperture nylon mesh (Tetko, Inc., Elmsford, N.Y.), and centrifuged for 5 min. The resultant pellet is resuspended in tap water to the desired cyst concentration and stored at 8°C. This procedure can be accomplished in 1 h yielding 30% cyst recovery, with purity acceptable for light microscopic examination and enumeration, and apparently undiminished cyst viability.

Three aliquots of *Giardia* cysts, purified from the same fecal specimen, were stored in unchlorinated tap water (pH6.8) at 37, 21, and 8°C, respectively. Cyst viability, evaluated by both methods, was assessed

*Table 5. Excystation of* Giardia *cysts exposed to temperature-varied HCl (pH 2.0) and transferred to temperature-varied HSP-3.*

| Acid temperature (°C) | HSP-3 temperature* (°C) | | |
|---|---|---|---|
| | 8 | 21 | 37 |
| 8 | 0 | 0.5 | 1.5 |
| 21 | 0 | 3.0 | 5.4 |
| 37 | 0 | 15.6 | 100.0 |

* Values represent percentage of excystation with reference to the control tube (HCl 37C, HSP-3 37C) which is arbitrarily 100%.

periodically for as long as the cyst suspension lasted, or until excystation and dye exclusion had decreased to zero for 5 days. The results of these experiments are summarized in Figures 1, 2 and 3. In every case, the eosin-exclusion test indicated higher levels of viable cysts than could be demonstrated by the excystation procedure. In cysts incubated at 21 and 37°C (Figs. 1 and 2) eosin-staining (indicating dead cysts) eventually reached 100% but not until several days (even weeks in some cases) after excystation indicated the same thing. With cysts stored at 8°C for 77 days (Fig. 3), eosin-staining indicated more than 80% viability while the excystation procedure indicated viability of less than 1%. If one considers the ability to excyst as a criterion of viability, then our results suggest that in vitro excystation is a more sensitive indicator of cyst viability than is eosin-exclusion. Furthermore, the results indicate that cysts which neither stained with eosin nor excysted were either dead, or alive but incapable of excysting.

*Giardia* cysts stored at 8°C had higher levels of excystation for a longer duration than those stored at 21 or 37°C. Cysts stored at 37°C never survived longer than 4 days and usually the percentage of excystation was greatly reduced even after 24 h (Figure 4). At 21°C, cyst survival ranged from 5 to 24 days. In all cyst suspensions examined from fresh fecal specimens and stored at 8 or 21°C, low initial levels of excystation were observed. This period of low excystation, varying from 2 to 7 days and followed by an increase in the level of excystation, suggests that a

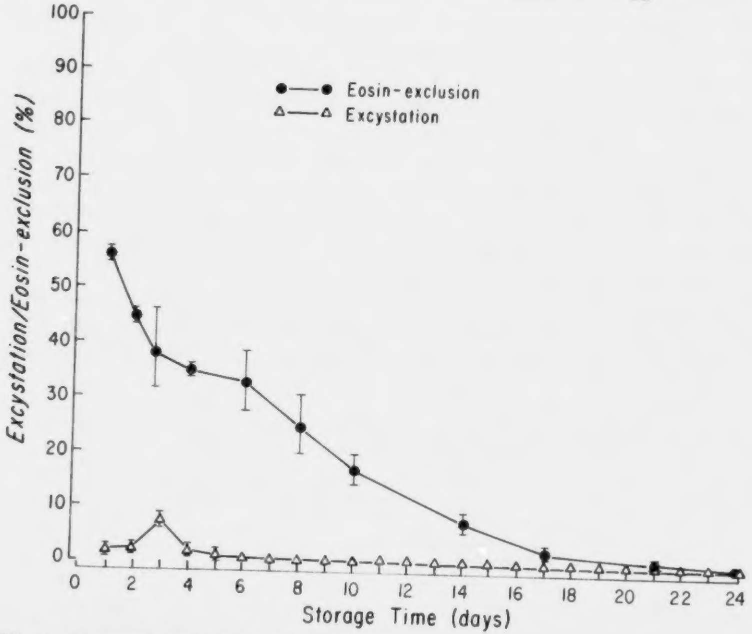

**FIG. 1.** ***Comparison of* Giardia *viability as determined by excystation and eosin-exclusion of cysts stored at 37°C.***

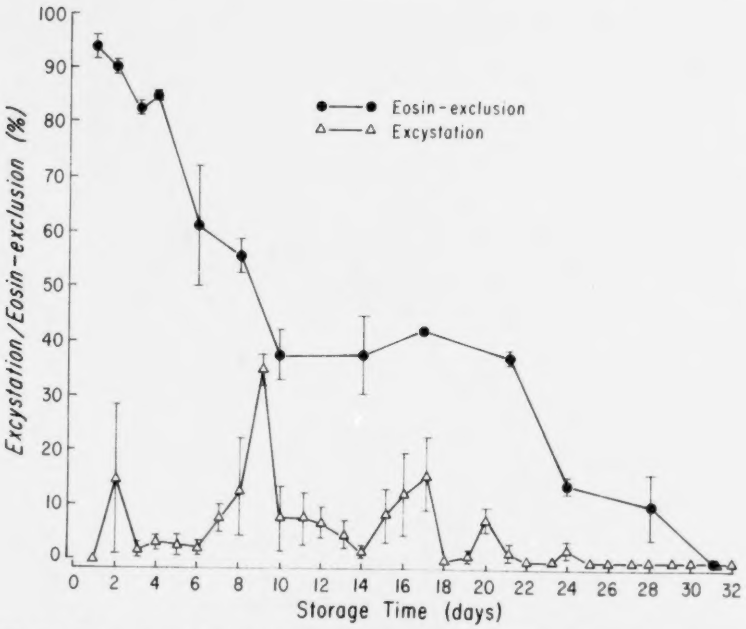

**FIG. 2.** *Comparison of Giardia viability as determined by excystation and eosin-exclusion of cysts stored at 21° C.*

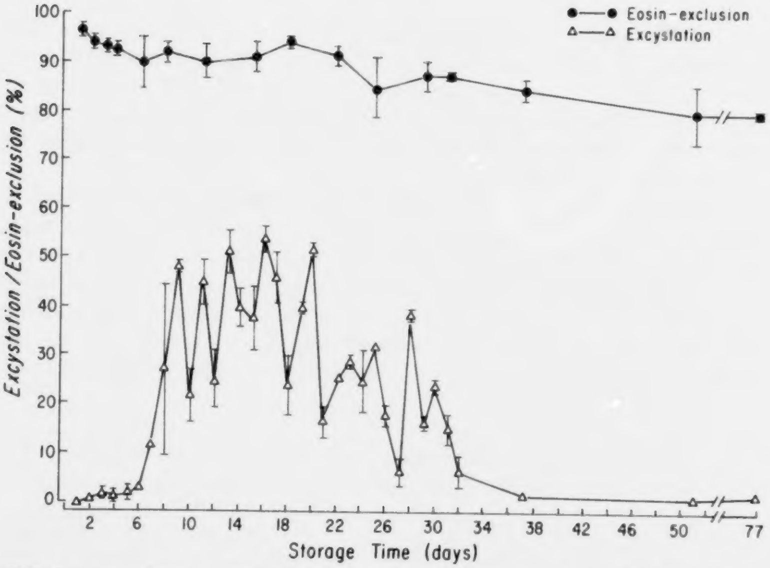

**FIG. 3.** *Comparison of Giardia viability as determined by excystation and eosin-exclusion of cysts stored at 8° C.*

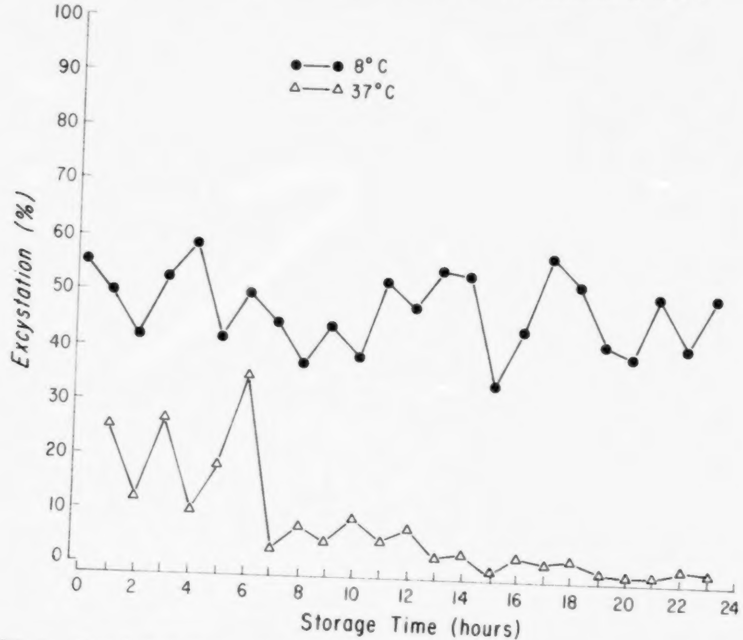

**FIG. 4.** *The effect of storage at 8 and 37°C on* **Giardia** *cyst viability as determined by excystation. Cysts were permitted to mature at 8°C for 1 week prior to beginning of the experiment.*

maturation period is required before *Giardia* cysts reach their full excystation potential. A maturation period of 800 h or more has been reported for *Acanthamoeba castellanii (18).* Even though Dutta and Jehan (19) found that they could induce *A. castellanii* excystation in less time than the previous workers, the cysts still required a maturation period of approximately 3 days.

In an attempt to assess the effect of freezing and boiling on *Giardia* cysts, 3 aliquots of the cyst suspension were placed at temperatures of 100, 8 (as control), and -13C, respectively, and viability determined periodically by the in vitro excystation procedure. Cysts subjected to 100°C were killed immediately (Table 6). Freezing cysts also greatly reduced their viability (Figure 5), but it is interesting to note that very low levels of excystation were observed even after low temperature storage for 14 days. This suggests that

*Table 6. Percentage excystation of* Giardia *cysts exposed to boiling water.*

| | Control (8°C) | Boiling time (minutes) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 5 | 10 | 15 | 20 | 25 | 30 |
| % excysting | 29.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

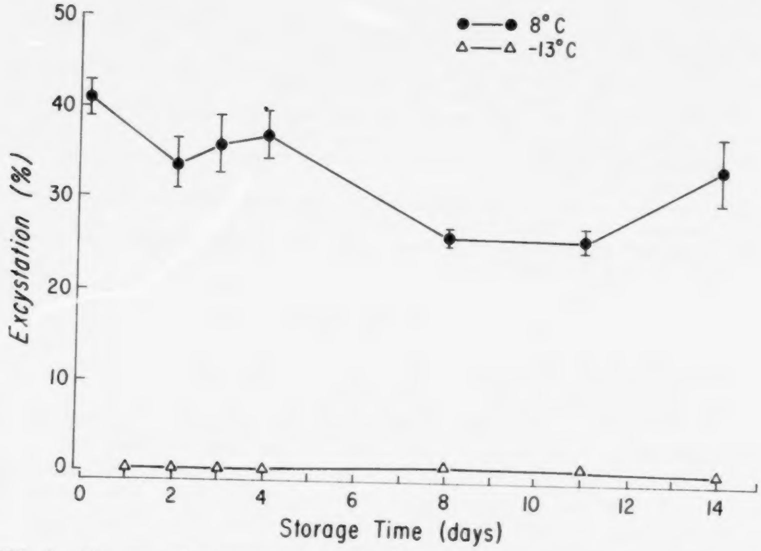

FIG. 5. *The effect of storage at 8 and -13°C on* **Giardia** *cyst viability as determined by excystation. Cysts were permitted to mature for 1 week prior to beginning of experiment.*

the freezing process itself and not the low temperature may be the principal factor responsible for cyst death.

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## Discussion

D. STEVENS: What strain of *Giardia* did you use?

A. BINGHAM: It was a human strain.

D. STEVENS: Have you looked at differences between *G. muris* and *G. lamblia* in terms of resistance of cysts to various physical factors?

A. BINGHAM: No. We only performed the excystation procedure with *G. muris,* and we had good success. Using pH 2 for approximately 30 minutes, we obtained about 50 or 60% excystation with *G. muris,* which is comparable to what we obtained with *G. lamblia.*

D. STEVENS: Have you compared excystation to viability as demonstrated by infectivity of the cysts?

A. BINGHAM: We have not yet. It seems like a difficult problem, but we may approach it in the future.

G. JACKSON: This is really a lovely series of experiments on excystation. Did you perform any experiments on encystation?

A. BINGHAM: When I initially started experiments in the lab, I made a few different attempts to induce encystation. I decided that excystation might be a more fruitful area and, fortunately, it was for my thesis. Thus far we have not had any success with our encystation attempts.

G. HEALY: I would like to add my congratulations for a beautiful piece of work. I am intrigued by the openings in the end of the cysts where the trophozoites come out. I do not remember seeing any electron micrographs indicating a weakness in the cyst in this area. Do the trophozoites always come out from the same area of the cyst?

A. BINGHAM: We have noticed some interesting things concerning that area of the cyst. We have not been able to determine which end of the cyst, if you can differentiate the ends, the trophozoite usually comes out of.

Fortunately we had the same donor for the entire period of our experiments. As this donor approached spontaneous remission we observed increasing numbers of odd-shaped cysts from which trophozoites attempted to excyst at the side of the cyst rather than through one end. Excystation was never successful in these cysts.

We believe this to be a type of abortive excystation and may be related to the immunological response of the host. The majority of viable-looking cysts in good preparations will emerge from one end; we do not know which end it is or if that makes any difference.

L. HIBBERT: Did you notice any thinning of the membranes on the end of the cyst just after your hydrochloric acid treatment? I was wondering if this might have been observed prior to your treatment with the hydrochloric acid.

A. BINGHAM: No. With the light microscope the most detailed thing that we have been able to see is the withdrawal of the trophozoite from the cyst wall and then penetration of the wall, and we have not been able to visualize what happens during that particular moment.

V. OLIVIERI: Could you give a more detailed description of your method of calculating per cent of excystation? Was it a method similar to what R.P. Stringer (*J. Parasitol.* 58:306, 1972) used for *Entamoeba histolytica*, counting empty versus full cysts?

A. BINGHAM: No, it was not. We found that counting empty *Giardia* cysts is more difficult and less accurate than counting trophozoites. I assume that with *Entamoeba histolytica* there is a great deal of activity and movement immediately after excystation. However, with *Giardia* we found that in most instances this is not the case. The trophozoites, once they have excysted, will settle down immediately near the cyst and complete the process of division, and usually they will stay there. With the cultures that we have initiated, we have noticed that the trophozoites will settle down in one spot and multiply there; they do not seem to move.

Another reason that we prefer trophozoites to empty *Giardia* cysts is that, particularly when there is a moderate amount of debris, empty cysts are sometimes difficult to see. On the other hand, the trophozoites have active flagella and will cause a great amount of stirring. In fact, they clear out large areas of debris around them with this flagellar motion, and are easily observed.

R. OWEN: Would you care to speculate how giardiasis can then occur in patients with achlorhydria?

A. BINGHAM: If we can bar the possibility that these people were already infected or that they may have somehow consumed trophozoites rather than cysts, then I would have to put forth the opinion that there is a large variability in the sensitivity of cysts to various pH's. There seem to be sub-populations of cysts within one fecal specimen with varying sensitivity and responses to pH and exposure time to acid. There may be a small percentage of cysts within any population of cysts which will excyst even under near neutral conditions. We have seen low level, maybe 0.1 or 0.2 per cent, excystation even in solutions which were close to neutrality, pH 6.2 to 6.8, and this may be one possible explanation for infection in these people.

F. GILLIN: I would like to know the approximate time course of the excystation sequence.

A. BINGHAM: With very active cysts in a good, viable preparation, we usually see excystation occurring within 10 to 30 minutes following transfer into a HSP-3 medium. The majority will be out within 10 to 15 minutes. They emerge very rapidly if they are going to emerge.

F. GILLIN: Have you tried to keep the emerged trophozoites growing in culture?

A. BINGHAM: Yes, we have. We have established trophozoite cultures from two humans and from monkeys axenically. We have also induced excystation with *Giardia* cysts from dogs, cats, rats and mice thus far, and we are very confident that we can probably do it with just about any cyst from any species of animal.

A. TOMBES: I have not seen any pores in the end of the cysts as you indicated. However, on *Isospora*, there is a very definite pore probably a half micron in diameter in one end of the cyst for the organism to come out. Could you comment further on the odd shaped cysts that you saw at different times?

A. BINGHAM: We saw cysts that ranged from the normal oval shape to square, teardrop, or dumbbell shaped. Most of the time we saw these odd shapes in preparations from this particular patient as he approached spontaneous remission of the infection. We assume that this may have something to do with the immune response.

From my recollection, the total viability with excystation in these preparations is much lower than with the normal looking cyst. We have had excystation as great as 60% with good batches of normal looking cysts, whereas with these other batches excystation normally is 5 per cent or less.

J. HOFF: I would like to add our congratulations on the quality and success of your research in this area. I am particularly happy since EPA has been funding this research for the last couple of years. This research really opens the way for addressing the applied type of question concerning water treatment, that is, regarding these organisms and disinfection. We look forward to good progress in this area in the future, particularly since you are involved in this work now.

In the study during which you stored the cysts for a period of 24 hours and over all showed no decline in viability there was considerable variation in the per cent excystation from one sample to another. Is that due to errors in counting? Does it indicate an extreme sensitivity to exposure to the acid? Do you have any idea what caused the variations?

A. BINGHAM: We are convinced that at least a portion of it is due to sampling variation since we are taking a relatively small sample. For most of the results that we have shown, we had counted maybe 1,000 cysts at one particular value. A total of 36,000 cysts were sometimes counted in one experiment, but even that was only a small percentage of the total population in a cyst preparation.

However, over weeks of storage we notice peaks of excystation followed by troughs which will last for several days and then a peak again, with as great as 30 or 40% difference between a trough and peak. I believe that this is related to something inherent in the cysts, as well as to sampling variation. In other words, maybe some cysts are ready to excyst at one particular time whereas others are not. I do not know the reason for this. There might be some kind of population effect there, possibly related to age. There may be some maturation that occurs in the gut. One group of cysts may remain in the gut for 2 days or longer, and are excreted mature and ready to excyst; another sub-population may be excreted rapidly and are a day or two less mature. So as a result, we see excystation peaks 1 or 2 days apart.

S. ERLANDSEN: You mentioned a latent time of approximately 2 to 7 days before excystation. What was the medium for storage of your cyst material? Were these cysts that were kept at refrigerator temperatures, and would then excyst after that latent period?

A. BINGHAM: The cysts were stored in tap water at 8° C and did excyst after a latent period.

S. ERLANDSEN: We are concerned with fecal droppings in some of these reservoir areas. In your attempts to examine feces, what type of cyst viability might you expect with material that is left out to essentially desiccate as we expect to take place in nature?

A. BINGHAM: When I began these experiments I initially did try leaving fecal specimens in the refrigerator and using portions as I needed them before we had developed a purification procedure. In my recollection the cysts did not survive as long as purified cysts. It seems to me that the cysts will last a little longer in water than they will in feces, and as far as desiccation goes, we have not done anything in that area.

# SESSION V — WATER TREATMENT TECHNOLOGY

*Chairman - David W. Liechty, Department of Social and Health Services, Olympia, Washington*

**Disinfection Resistance of *Giardia* Cysts: Origins of Current Concepts and Research in Progress**

**J. C. Hoff**

**Water Filtration Techniques for Removal of *Giardia* Cysts and Cyst Models**

**G. S. Logsdon, J. M. Symons, R. L. Hoye, Jr.**

# Disinfection Resistance of *Giardia* Cysts: Origins of Current Concepts and Research in Progress

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## ABSTRACT

Our current concept of the resistance of *Giardia lamblia* cysts to disinfection, as applied in treatment of drinking water, is that the cysts are highly resistant and that their control by disinfection is not feasible. The origins of this concept appear to be based on early disinfection studies and on their similarity to another pathogen, *Entamoeba histolytica* which has been shown to be highly resistant to disinfection. The results of more recent studies indicate that the early studies in which *E. histolytica* and *G. lamblia* cyst viability was determined by dye exclusion provided results indicating erroneously high disinfection resistance. Therefore, the early disinfection studies should be disregarded. The application of a recently developed in vitro excystation method to *Giardia* disinfection studies and the current *Giardia* disinfection research program are discussed.

The recent occurrences of waterborne outbreaks of giardiasis have resulted in a recognition of our lack of knowledge of the effects of water treatment processes on the etiologic agent, *Giardia lamblia*. The most important single water treatment unit process for the control of pathogenic microorganisms is disinfection. The initiation of chlorination in the treatment of drinking water in the early part of this century caused a dramatic reduction in the occurrence of cholera, typhoid fever and other waterborne diseases.

Because it has been considered unimportant as a waterborne pathogen, *G. lamblia*, in contrast to the enteric bacteria, viruses and other pathogens, has received little study with regard to disinfection. Based on a few early studies suggesting that the cysts were extremely resistant to disinfectants and because of its similarity to another protozoon, *Entamoeba histolytica*, *G. lamblia* is currently regarded as highly resistant to chlorine.

The purposes of this paper are to review the earlier literature, discuss the present state of our knowledge regarding the disinfection resistance of *G. lamblia* and *E. histolytica* cysts and outline an approach for generating disinfection information on *G. lamblia* that will be useful in assessing the adequacy of disinfection as a water treatment unit process for preventing waterborne outbreaks of giardiasis.

## *G. LAMBLIA* DISINFECTION — SOURCES OF CURRENT PERCEPTIONS

As indicated above, the prevailing concept conveyed in the current literature relating to the *G. lamblia* cysts is that they are extremely resistant to chlorine. A statement to the effect that "*Giardia* cysts resist 0.5% chlorinated water for 2 to 3 days" appears in reference texts (1,2) and in at least 2 recent review papers (3,4).

To those familiar with and involved in drinking water disinfection, a chlorine concentration of 0.5% translates into an available chlorine level of well over 1000 mg/l. Chlorine levels used in water treatment are usually on the order of 2 to 3 orders of magnitude lower than this with contact times of 30 min to a few hours. Thus, the concept conveyed is that the cysts are extremely resistant to disinfection and impossible to control by disinfectant concentrations and contact times used in water treatment. Unfortunately, the source of this statement was not cited in any of the reports.

A search of the early literature on protozoan cyst inactivation indicates that the source of the statement may have been a paper published by Mills et al (5). As part of a study of contamination of fruits and vegetables by microorganisms these authors examined the resistance of a number of protozoan cysts, including *Giardia intestinalis (lamblia)* to chlorinated water, alcohol, and boiling. The information conveyed by their results is that *G. intestinalis* and *E. histolytica* cysts WERE killed by exposure to 0.5% chlorinated water for 2 to 3 days. However, Liu (6) cited the earlier work as follows: "According to Mills, Bartlett and Kessel (1925) 0.5 per cent chlorinated water will kill most kinds of bacteria but not the protozoan cysts in 2-3 days." It may have been this unfortunate misstatement repeated in subsequent publications that resulted in the conveyance of the concept of extreme resistance of *G. lamblia* cysts to chlorine.

### Viability Based on Vital Staining

Although Penfold et al (7) apparently had succeeded in demonstrating *E. histolytica* viability by microscopically observing excystment in a nutrient medium, all of the early studies on the effects of environmental conditions on survival of protozoan cysts were carried out using vital staining as an indicator of cyst viability. In this test, eosin was usually used for staining. Cysts which remained colorless when exposed to eosin were considered viable while those penetrated and stained by the dye were considered dead. According to Boeck (8), the basis for the use of this type of test as a measure of protozoan cyst viability was the lack of other reliable measures of viability such as in vitro excystment or animal infectivity. However, it is evident that the validity of staining as a measure of viability was questioned by many early workers. Both Boeck (8) and Root (9) quoted Wenyon and O'Connor who had also used this test in studies published in 1917 as follows:

> "It seems, therefore, clear that the eosin-staining cells are dead though it may be argued that others which do not stain may be dead also or, at any rate, noninfective. Still, if we accept the eosin test as a criterion and regard all unstained cysts as living, the error in judgement will be on the safe side."

In a more recent publication on the effects of disinfectants on *E. histolytica* and *G. lamblia* cysts, Cerva (10) used staining with eosin for measuring cyst viability after exposure to disinfectants. He reported that a 1 to 3% solution of chlorine or chloramine killed (as determined by vital staining) only 10% of *G. lamblia* in 30 min to 24 hours. A 10% solution killed 30 to 40% in 1 h in 6 of 10 experiments. Similar results, showing extremely high resistance to chlorine, were obtained in comparative studies on *E. histolytica*. However, *E. histolytica* disinfection studies conducted by Chang and Fair (11) using culture technics to determine viability indicated that these cysts were inactivated by much lower levels of chlorine, thus demonstrating the lack of correlation between vital staining and culture for determining cyst viability. Although data were not shown, Chang and Fair reported that staining of cysts with eosin was grossly unreliable as a criterion of cyst death as determined by cyst culture. In a later publication, Chang (12) stated:

> "I have observed from time to time that cysts were found to be nonviable by the culture method but 40 to 70 percent failed to take the stain (EOSIN). Also, an examination of cysts stored in a cold room 42 to 50° F, for four to five months after the period of viability showed that 30 to 40 percent failed to be stained. It is felt that while the nuclei of cysts are no longer viable, the cystic wall may resist the infusion of dyestuff into the cyst for a long time."

In their work on the effects of chlorine on *E. histolytica* cyst viability Brady et al (13) also reported that in preliminary experiments:

> "The degree of correlation between the indicated survival of cysts by the eosin-staining technic and the actual survival by ability to establish cultures was not close enough to permit the use of the former as a criterion."

The report of Bingham et al (14) has confirmed and quantified the differences between measurement of *G. lamblia* cyst viability by eosin staining and measurement by the in vitro excystation technic they have developed (15). Their results indicate that eosin exclusion consistently indicates higher cyst viability than can be shown by in vitro excystation.

If the above detective work is correct in tracing the source of current information indicating that *G. lamblia* cysts can resist 0.5% chlorinated water for 2 to 3 days, the concern generated by such statements should be dispelled since the results on which it is based were both misstated and were obtained using viability measurements that have been shown to provide erroneous results.

## *E. histolytica* Resistance to Disinfectants

As indicated previously, a second source of our current impression that *G. lamblia* is highly resistant to disinfection stems from its similarity to *E. histolytica*. Both are protozoons, existing in two stages. One stage is a cyst form in which the organism exists in a nonmotile state inside a more or less impervious protective cyst wall which enables its survival outside the host in unfavorable environmental conditions. In the other stage the organisms are actually motile and extremely fragile, an amoeba in the case of *E. histolytica* and a flagellate in the case of *G. lamblia*.

Development of culture media for the cultivation of *E. histolytica* (16) and of a cultural encystment method (17) coupled with increased interest in amebiasis, generated by several waterborne outbreaks in the U.S. and military needs associated with stationing troops in tropical countries, stimulated extensive disinfection studies on the cysts of this protozoon. These studies employed a variety of disinfecting agents (chlorine, chloramines, iodine, bromine, ozone) under a variety of conditions and resulted in the formation of a large body of disinfection information on *E. histolytica.*

In 1956, the National Research Council (18) prepared recommended chlorine and chloramine bactericidal and cysticidal residuals for drinking water treatment by the armed forces under field conditions. The recommended levels were based on a 30 min contact period and provided for different residual levels at various water pH's and temperatures. The cysticidal recommendations were based on studies by Chang and Fair (11) and Chang (12), as well as results of other unpublished studies most of which had been conducted by Chang and others at the Department of Sanitary Engineering and School of Public Health at Harvard University. Table 1 was prepared by Palin (19) based on the National Research Council Report (18). The results indicate that levels of free chlorine needed to inactivate *E. histolytica* cysts at temperatures of 22 to 25°C at low pH are reasonably attainable in water treatment practice. However, at higher pH's at 22 to 25°C and at any pH at temperatures of 2 to 5°C, relatively high levels of chlorine that would be considered impractical are necessary. Chloramines, which are much weaker biocides than free chlorine, were considered impractical for cyst control. These results are consistent with many other bacterial and viral disinfection studies, reflecting the greater disinfecting efficency of chlorine and other oxidants at higher temperatures and also the influence of pH on disinfection rates through its influence on the chemical disinfectant species present.

Our present concept of the disinfection resistance of *E. histolytica* cysts stems largely from the research on which the National Research Council report was based. Thus, White (20) stated:

> "The best protection against this disease (amoebic dysentery) is removal of the organisms from water supplies by filtration. They are large enough to be trapped by sand filters, and require high doses of free residual chlorine (10 ppm) and long contact time (one hour or more) to effect disinfection."

*Table 1. USPHS minimum cysticidal and bactericidal residuals*[a]
*(After 30 minutes contact)*

| pH Value | Free Chlorine, HOCl + OCl⁻ | | | Combined Chlorine, NH2Cl |
|---|---|---|---|---|
| | Bactericidal 0-25°C | Cysticidal 22-25°C | Cysticidal 2-5°C | Bactericidal 0-25°C |
| 6.0 | 0.2 | 2.0 | 7.5 | 2.0 |
| 7.0 | 0.2 | 2.5 | 10.0 | 2.5 |
| 8.0 | 0.2 | 5.0 | 20.0 | 3.0 |
| 9.0 | 0.6 | 20.0 | 70.0 | 3.5 |

[a] Concentration required for inactivation of 99.999% of cysts. Adapted from (19).

Subsequently, Stringer (21) developed a method for determining *E. histolytica* cyst viability based on microscopic observation of full and empty cyst walls following incubation of cysts in excystation medium. He pointed out that this viability assay offered a simple and reliable method for determining the percentage of living cysts in a cyst population and provided more quantitative results than the methods based on cyst growth in liquid medium that had been used in previous *E. histolytica* disinfection studies.

Using this method, Krusé (22) and Stringer and Krusé (23,24) reexamined the effects of chlorine, iodine, and bromine on amoebic cysts. They used a standard exposure period of 10 min at 30°C over a range of pH 5 to 10, with varying concentrations of each disinfectant under both clean and extraneous disinfectant demand conditions. It is difficult to compare their results with those in Table 1 based on the National Research Council's analysis because of the different contact periods (10 min vs 30 min) and the endpoints chosen (50 to 99.9% inactivation vs 99.999%). The 30°C temperature used by Stringer and Krusé (23,24) was also considerably higher than in the previous studies. However, Krusé (22) cited the National Research Council recommendations for cyst chlorination at pH 7.0 as in agreement with the results of his studies at this pH. Although the differences in experimental conditions used make it difficult to correlate the results, they appear to be in fairly good agreement and convey the concept that *E. histolytica* cysts are among the most chlorine resistant waterborne pathogens known. Depending on water plant operating conditions with regard to water temperature, pH, and disinfectant contact time, it appears that *E. histolytica* cyst inactivation would be practicable under some conditions but not under others.

Thus, if *E. histolytica* cysts are a good model for indicating the disinfection resistance of *G. lamblia* cysts, the concept that disinfection cannot be relied on for preventing waterborne transmission of giardiasis is well founded. Unfortunately, their validity as a model is not known and it would appear that the only way the model can be validated is to conduct disinfection research on *G. lamblia*.

## CURRENT *G. LAMBLIA* DISINFECTION RESEARCH

The development of an in vitro excystation method for *G. lamblia* and its successful application in the viability studies presented at this symposium (14) indicate that disinfection studies on *G. lamblia* cysts are feasible.

In our laboratory, *G. muris* is being used as a *G. lamblia* model and chlorine inactivation studies on this species have been initiated using the in vitro excystation method. The cyst suspending medium used has been 0.05 M phosphate buffer or low turbidity, clean, natural water. The natural water was filtered through an 0.22 m porosity filter to remove particulates. Both the buffer and natural water were rendered chlorine demand-free by addition of excess chlorine, storage for 24 hours and destruction of the remaining chlorine by exposure to ultraviolet light. This process satisfied any chlorine demand caused by impurities in the water and thus aided in maintaining a constant chlorine residual during the experiment.

Prior to each experiment, chlorine was added to a previously refrigerated suspending medium to the desired level (1 mg/l), the pH was adjusted, and 1 liter was placed in each of four 1 liter beakers. These were placed in a 5° C circulating water bath. The contents of each beaker were continuously stirred by overhead stirrers. The chlorine in the cyst control beaker was neutralized by the addition of 1 ml of 10% sodium thiosulfate. At time zero purified cysts calculated to provide a concentration of 100 to 200 cysts/ml were added to each of the stirred beakers. At appropriate time intervals, sodium thiosulfate was added to each beaker, the beaker removed from the water bath and the contents filtered through 5 ˙m porosity 47 mm polycarbonate filters to separate the cysts from the suspending medium. The cysts were washed from the filter surface into a 15 ml conical centrifuge tube using 1 ml of water. The cysts were exposed to pH 2 HCl for 30 min to trigger excystation, reconcentrated by centrifugation and incubated in excystation medium for 1 h at 37° C in sealed glass coverslip preparations and examined for full and empty cysts and the presence of trophozoites as described by Bingham and Meyer (14).

The results of these studies are shown in Table 2. Although the data are preliminary, they do indicate that this approach can be used to provide disinfection data. The differences in *G. muris* percent excystation after exposure to 1 mg/l free residual chlorine at pH 7.6 and 9.0 are consistent with the well known decrease in disinfection efficiency of free chlorine as pH increases because of the predominance of hypochlorite ion ($OCl_\pi$), a weak disinfectant chemical species, at higher pH's. The results indicate that the suspending medium used in cyst disinfection studies may be an important factor. In bacterial and viral disinfection studies 0.05 M phosphate buffer is conventionally used to insure pH stability. In one of the preliminary experiments, plasmolysis, as indicated by shrinkage of the cytoplasm from the cyst wall, occurred when the cysts were reconcentrated from 0.05M phosphate buffer and placed in the excystation medium. The low excystation levels in nonchlorinated control cysts suspended in phosphate buffer also indicated that this may be a problem. Higher cyst control excystation was obtained when a natural water was used.

*Table 2. Preliminary experiments on* Giardia *cyst inactivation by chlorine*
(5°C, 1 mg/l free residual chlorine)

| Cyst Species | pH | Suspending Medium | Percent Excystation | | | |
|---|---|---|---|---|---|---|
| | | | Positive Control | Chlorine Exposure Time (Min) | | |
| | | | | 10 | 20 | 30 |
| *G. muris* | 6.0 | .05M $PO_4$ | 0 | 0 | 0 | 0 |
| *G. lamblia* | 7.0 | .05M $PO_4$ | 2.5[a b] | 0[a] | 0[a] | 0[a] |
| *G. muris* | 7.6 | water | 22 | 0[b] | 0 | 0 |
| *G. muris* | 9.0 | water | 21 | 20 | 6 | 7 |

[a] Cytoplasm shrinkage observed
[b] Incomplete excystation? Blebs observed

The results of 1 preliminary experiment designed to determine the effect of suspending media on excystation are shown in Table 3. The results bear out the initial observations made in the disinfection experiments and indicate that the suspending medium used in *Giardia* disinfection studies can affect the results obtained.

*Table 3. Preliminary observations of effects of suspending medium on* G. muris *cyst viability*[a]

| Suspending Medium | pH | Percent Excystation | Cytoplasm Shrinkage | Trophozoite Activity |
|---|---|---|---|---|
| 0.5M $PO_4$ | 6.0 | 7 | + | ± |
| 0.5M $PO_4$ | 7.0 | 4 | + | – |
| 0.5M $PO_4$ | 8.0 | 6 | + | – |
| Water[b] | 6.0 | 9 | – | ± |
| Water[b] | 7.0 | 15 | – | ++ |
| Water[b] | 8.0 | 20 | – | ++ |

[a] Stored 1 hr., 5°C w/ stirring at indicated pH

[b] Clean natural water, filtered (0.22 μm)

It is also possible that the cyst purification procedure involving concentrating the cysts on 0.8 M sucrose by centrifugal force could osmotically damage the cysts. The questions of optimum time in the infectious cycle for collection of cysts from the infected animals, optimum time for separation of the cysts from the fecal material, and whether or not cysts undergo a maturation process need to be answered. In order to provide reliable disinfection information, cysts in their optimum state of resistance and infectivity should be used in disinfectant exposure studies. In this regard it is interesting to note that Kruse[1] (22) reported that *E. histolytica* cysts produced in vitro using an encystment medium were less resistant to inactivation by chlorine than cysts produced in monkeys. Thus, while it would appear that development of a *G. lamblia* encystment medium would be advantageous in providing a reliable continuing supply of cysts for experimental work, the results of disinfection studies using culturally produced cysts might not accurately indicate the resistance of naturally produced cysts.

In addition to the in vitro disinfection studies on *G. muris*, limited disinfection studies using mouse infectivity assays for measuring viability also are being conducted by Dr. Stevens group at Case Western Reserve University. The results of these studies should make it possible to relate the validity of in vitro results to actual host infectivity. The difficulties in obtaining a continuing and consistent supply of *G. lamblia* cysts make the use of readily obtainable cysts such as those of *G. muris* especially attractive. It is expected that, through the above efforts, disinfection information will become available for use in devising water treatment processes that will effectively prevent future waterborne giardiasis outbreaks.

## SUMMARY

Analysis of the results of early studies on *Giardia* cyst inactivation by disinfectants indicates that the results of these studies should be disregarded since the method used for viability determination (dye exclusion) has been shown to give inaccurate results. The recently developed in vitro excystation method appears promising for disinfection research on *Giardia* cysts and research designed to provide disinfection information on this protozoon is in progress.

## ACKNOWLEDGMENT

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## Discussion

L. LEONG: Are you aware of any research into the effects of ozone on *Giardia* cysts?

J. HOFF: There has been some work on the effects of ozone on *Entamoeba histolytica*. I am not aware of any that has been done on *Giardia lamblia*.

M. WOLFE: It may be appropriate to recall that in the studies done during World War II on field disinfection of small bodies of water, it was conclusively shown that the amount of chlorine released by Halazone tablets was inadequate to kill amebic cysts. Then the Globaline iodine tablet was produced which did kill amebic cysts. Globaline is not readily available on the commercial market, but there is something called Potable-Aqua tablets (Badger Pharmacal, tetraglycine hydroperiodide) that contain iodine. We are ignorant of what the effects of iodine tablets would be on *Giardia* at this point until your studies are carried further. I think all we can do is analogize to the situation that occurs with *Entamoeba histolytica*.

My current recommendation for travelers and to people hiking in the Rocky Mountains, who may want to drink stream or other untreated water, is to use iodine water purification tablets or tincture of iodine solution rather than to rely on Halazone tablets. Some doctors and others are making recommendations to travelers to use chlorine tablets, but I think it is important to emphasize that iodine appears to be superior at this point. Hopefully, the same findings with *Giardia* as have been shown for *Entamoeba histolytica* will be confirmed. At this time I think it is wise to use iodine for field disinfection.

J. HOFF: Assuming the similarity of *G. lamblia* to *E. histolytica*, I think that is probably a good recommendation.

# Water Filtration Techniques for Removal of Cysts and Cyst Models

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## ABSTRACT

Because outbreaks of waterborne giardiasis have been documented only in recent years, information on the removal of *Giardia* cysts by water filtration is limited to filtration failures associated with outbreaks.

Filtration for removal of *Entamoeba histolytica* cysts was studied in the 1930's and 1940's. Granular media filtration was effective for *E. histolytica* cyst removal if the water was properly preconditioned. Minimum preconditioning required was effective coagulation. Sedimentation and diatomaceous earth filtration also were effective for *E. histolytica* cyst removal.

Recent experiments, although limited, showed that *Giardia lamblia* cysts were removed by diatomaceous earth filtration. Preliminary laboratory results also suggest that coagulation and granular media filtration will remove *Giardia* cysts.

Both previous work with granular media filtration and present work using 9 μm radioactive microspheres as cyst models show that good plant operating procedures are necessary if water filtration plants are to be an effective barrier against the passage of cysts into drinking water. Water must be properly coagulated before granular media filtration. Diatomaceous earth filters should be precoated with 91 g (0.2 lb) of diatomite/0.1 $m^2$($ft^2$) of filter surface, and body feed must be added to maintain the integrity of the filter cake. Conscientious, high quality filter plant operation is a key factor in providing public health protection against waterborne giardiasis.

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Outbreaks of waterborne giardiasis have been occurring more frequently in recent years. This trend is a cause for concern among public health, environmental, and water utility officials. This paper will discuss some possible engineering solutions.

## THE PROBLEM

Kirner et al in a discussion of a giardiasis outbreak at Camas, Washington, stated "Chlorination in the manner being practiced by the city was either ineffective in disinfecting the raw-water supply, where the infective organism was concerned, or the failure of disinfection equipment allowed enough organisms to survive to cause a problem" (1). They also stated that turbidity and coliform count alone were not adequate parameters for assessing the biological quality of filtered water.

For situations in which monitoring methods are not adequate to insure production of potable water, the use of certain water treatment techniques may be appropriate. This concept is included in the Safe Drinking Water Act. The efficacy of chlorination at Camas was uncertain, and other cysts, such as *E. histolytica* are known to be much more resistant to chlorination than are coliforms (2). Therefore, removal of *Giardia* cysts by filtration should be a practical approach to providing safe drinking water where the threat of *Giardia* contamination of water sources exists. This paper will review literature on water clarification for cyst removal and present recently obtained data on the subject.

## PRINCIPLES OF FILTRATION

Water filtration has been practiced for many years. The concept of conventional water treatment in which coagulation, flocculation and sedimentation are used to condition water for filtration, was studied carefully by G. W. Fuller at Louisville, Kentucky in the 1890's. The final clarification step in conventional water treatment is filtration through granular media.

Since World War II, design engineers have tended to use multi-media (dual media or mixed media) filters rather than the sand filters studied by Fuller. By carefully selecting media sizes and densities, engineers can design filter beds with the largest type of media at the top of the filters, where the pretreated water enters the porous bed. This facilitates storage of particulates and results in longer filter runs, or ability to operate filters at higher rates, or both.

Numerous water sources in the United States are low in suspended solids. When the color of such waters is low, the coagulant chemical dose needed to prepare the water for effective filtration is low. In such circumstances, sedimentation may be very ineffective for removal of particulate matter even though the filtration process is very efficient. Beard and Tanaka (3) demonstrated this by the use of particle counting. If the particulate content of a coagulated water is low, filtration without sedimentation (direct filtration) may be used to remove particulates from the water. Proper coagulation is just as essential in direct filtration as it is in conventional treatment.

Some low-particulate waters are treated by diatomaceous earth (DE) filtration. The sizes of the DE particles used as filter medium vary, but the range can be from several micrometers to forty or fifty micrometers. Mechanical straining is the mechanism of removal for particles in the size range of *E. histolytica* and *Giardia* cysts. Coagulation is generally not employed in DE filtration.

## LITERATURE REVIEW

A review of literature revealed no research on water filtration for *Giardia* cyst removal. This is not surprising because the *Giardia* organism was not implicated as a cause of waterborne disease until recently. The hazards of waterborne amoebiasis have been known for decades, so both before and during World War II water filtration studies for removal of *E. histolytica* cysts were carried out.

## Granular Media Filtration

Granular media filtration was in use in numerous water plants in the 1930's when an amoebic dysentery outbreak occurred in a Chicago hotel, so investigations of the capabilities of this process for cyst removal were performed.

### Chicago Experimental Filtration Plant Research

After the Chicago outbreak occurred, experiments for removal of *E. histolytica* cysts from water were carried out at the Experimental Filtration Plant operated by the Chicago Department of Public Works. In preliminary laboratory experiments, 4.1 cm (1.5 in) diameter filter columns were used. Tests with these small columns showed complete removal of *E. histolytica* cysts from water by coagulation and rapid sand filtration (4).

Further testing with a pilot plant filter having a surface area of $0.93 m^2$ (10 sq ft) took place in 1935 and 1936 (5). In this research, cysts were added to coagulated water that was then treated by direct filtration at 7.7 lpm/$0.1 m^2$ (2 gpm/$ft^2$). During the filter runs, which ranged in length from 4 to 14 hours, samples were taken at regular intervals. The samples were concentrated in a Foerst centrifuge, and the sediment was examined in a Rafter counting cell. Five tests for recovery of cysts by the centrifuge technique gave ratios of recovered/original ranging from 0.71 to 1.18.

Analysis of the filtered water showed the test filter was effective. A total of 433 l of filtered water was centrifuged and the sediment was examined for cysts. On the basis of the total volume of water filtered and the total number of cysts applied, 178,000 cysts would have been dosed in 433 l. Only 4 cysts were found in the filter effluent. This indicates a removal efficiency exceeding 99.99%. Baylis et al (4) concluded that filtration of water through rapid sand filters, in the manner then extensively used in the United States (2 gpm/$ft^2$), was an effective means of removing *Entamoeba histolytica* cysts from water.

### War Department Research, 1943-1944

The need to provide water free of *E. histolytica* cysts in World War II resulted in a cooperative filtration research program involving The Engineer Board, U.S. Army, Fort Belvoir, Virginia, and the National Institute of Health, U. S. Public Health Service. A report of this work was prepared by the War Department (6), but the report was not widely circulated. This paper will present a summary of that report, subsequently referred to as the Army report.

An apparent contradiction develops when Baylis' conclusion that rapid sand filtration was successful is compared to the Army report conclusion that the complete removal of *E. histolytica* cysts was not accomplished when the U. S. Army Portable Purification Unit sand filter was operated at flow rates practical for field use. H. H. Black, Chief of the Water Supply Equipment Branch that prepared the Army report, briefly discussed sand filters in a paper on diatomite filtration, stating, "The need for better filtration equipment arose from the failure of sand filters to remove cysts of *Entamoeba histolytica* at the high rates of filtration employed in the field"

(7). How Baylis and Black reached different conclusions about rapid sand filtration can be understood if the objectives of treatment and process operating conditions are considered for each case.

Operating conditions, and possibly water quality goals, were less demanding at Chicago. The filter there was operated at 7.7 lpm/0.1m² (2gpm/ft²) rather than 24.6 or 36.6 lpm/0.1m² (6.4 or 9.5 gpm/ft²) as was done in the Army experiments. Cysts in raw water at Chicago ranged from 362 to 2370 per gal. Cyst concentrations for Army tests involving coagulation and filtration ranged from 2300 to 7000 per gal. In some of the Army tests cysts were dosed on a batch basis with 1 to 3 million cysts being added in 20 to 30 seconds. Whereas 4 cysts in 433 liters was acceptable filter performance to Baylis, Black apparently sought total removal of the cysts from the treated water. The Army report data on sand filtration are summarized in Tables 1 and 2. In Baylis' filtration studies the 433 l that contained 4 cysts in the filter effluent would have had 178,000 in the influent (99.9978 percent reduction). This gives 22 cysts passing the filter/million applied, a much better result than 250/million applied, the best result obtained with the Army Portable Water Purification Unit.

Although the Chicago experiment filter performed better than the Army portable unit, for the circumstances relevant to Baylis' evaluation of the sand filter, both his data and the best Army data show substantial reductions of cysts. A key factor is the expected concentration of cysts in the raw water. Cyst concentrations in a grossly contaminated small jungle stream or pond, in an area where amoebiasis was endemic, would probably be much greater than cyst concentrations in a lake or river in the USA.

Army equipment had to be able to purify any water that might be found in field conditions, whereas water treatment plants built in the USA are supposed to be located so that grossly contaminated water does not reach the plant intake. As a result of the differences in water quality standards domestic water filtration plants would not have to have the performance capabilities of the Army portable units. Whether or not sand filtration was deemed effective must have been in some measure a reflection of expected raw water quality.

Tables 1 and 2 show the influence of good operating practice on cyst removal. Tests I and VI (A and B) involved filtration of uncoagulated water. Test II was performed with poorly coagulated water. For these tests, the numbers of cysts passing the filter/million applied were much higher than for other tests with good coagulation and comparable experimental conditions.

## Diatomite Filtration

In the United States, the Army took the lead in developing diatomaceous earth filtration for drinking water with a research and development program during World War II. This work is described in the Army report (6). A discussion of DE filtration principles and a summary of bacteriological data developed in the project was also published by Black and Spaulding (7). However, this paper, published in 1944, merely mentioned a need to remove

*Table 1. Results of experiments with Army portable water purification unit (sand filter)*

| Experiment Number | Quality of Coagulation | Filtration Rate gpm/ft$^2$ | Calc. Number of Cysts per Gal Influent | Calc. Number of Cysts per Gal Effluent | Cysts Passing Filter per Million Applied | Percentage Reduction |
|---|---|---|---|---|---|---|
| I | None | 9.5 | 2100 | 250 | 119,000 | 88.09 |
| II | Poor | 9.5 | 6300 | 240 | 38,000 | 96.19 |
| III | Good | 9.5 | 2300 | 9 | 3,900 | 99.61 |
| IV | Good | 6.35 | 5000 | 4.5 | 900 | 99.91 |
| V | Good | 6.35 | 7000 | 10 | 1,400 | 99.86 |
| VI-B | None | 6.35 | 838 | 165 | 197,500 | 80.30 |

*Table 2. Results of experiments with Army portable water purification unit (sand filter). Operation at reduced rate following coagulation and sedimentation*

| Experiment Number | Pretreatment | Settling Time (Min) | Filtration Rate gpm/ft² | Calc Number of Cysts per Gal Influent | Calc Number of Cysts per Gal Effluent | Cysts Passing Filter per Million Applied | Percentage Reduction |
|---|---|---|---|---|---|---|---|
| VI-A | None | 90 | — | 10,000 | 838 | 83,800 | 91.62 |
| VI-B | — | — | 6.35 | 838 | 165 | 197,500 | 80.31 |
| | | | | Combined Results — | | 16,500 | 98.35 |
| VII-A | Alum + Soda Ash | 120 | — | 3,800 | 5.4 | 1,400 | 99.86 |
| VII-B | | — | 6.35 | 5.4 | 0.95 | 176,000 | 82.41 |
| | | | | Combined Results — | | 250 | 99.975 |
| VIII-A | Alum + Soda Ash | 75 | — | 3,800 | 56 | 14,700 | 98.53 |
| VIII-B | | | 6.35 | 56 | 2 | 35,700 | 96.43 |
| | | | | Combined Results — | | 526 | 99.947 |

cysts of *E. histolytica* without giving actual test results. Experimental data can be found in the Army report.

Experiments on diatomaceous earth filtration for cyst removal were carried out with a variety of DE filter systems, ranging from devices to provide batch filtration of as little as 3.8 l (1 gal) of water to equipment capable of filtering approximately 77 lpm (20 gpm). Data from various parts of the Army report have been combined and summarized in Table 3. This table includes data calculated by the present writers through the use of other original data.

Newly calculated data include all of the data for the ratios of cysts recovered from filtered effluent to cysts in the same volume of filter influent, as well as some data on filter aid usage and flow rates. Some data were not available in the Army report so no values are given in certain instances in Table 3.

Data in Table 3 do support the conclusion the DE filtration could remove essentially all cysts. The largest number of cysts recovered, 7, resulted from using contaminated water (225,000 cysts in 35 gal) to precoat the filter. Clean, filtered water should always be used for precoating DE filters.

## CURRENT RESEARCH

Past research on cyst removal was related to treatment for *E. histolytica* cysts. Research results demonstrated that the cysts could be removed and these results suggested that filtration also should be effective for reducing the concentration of *Giardia* cysts in water. To demonstrate this specifically, a program of filtration research with *Giardia* cysts and cyst models is being conducted.

### Diatomaceous Earth Filtration Research

The Drinking Water Research Division's pilot plant facilities include a DE filter, described fully in a Johns-Manville technical bulletin (8). This 0.1m$^2$(1 ft$^2$) filter was used to perform treatment studies involving *Giardia lamblia* cysts and radioactive bead cyst models. The test results, presented more fully elsewhere (9,10), are summarized briefly in this text.

#### Experimental Methods

The DE test filter was set up for operation as a pressure filter with a slurry feeder for body feed. The 0.1m$^2$(1 ft$^2$) filter was operated at 3.8 lpm (1 gpm). The filter system is shown in Fig. 1. The operation of the DE filter consisted of three steps: precoating, filtration, and backwashing.

A diatomaceous earth slurry was prepared by adding the desired weight of DE to tap water in the precoat tank and mixing the contents of the tank during precoating. The weight of diatomite added to the precoat tank was used to calculate precoat thickness expressed as pounds per square foot. The slurry was recirculated until the water in the precoat tank appeared clear and free of diatomite.

During the filtration process, a small amount of diatomaceous earth was continuously added to the raw water in order to maintain good hydraulic characteristics of the precoat filter cake. Without the body feed, the surface

*Table 3. Summary of operating conditions and cyst data resulting from U.S. Army tests with diatomaceous earth filters.*

| Test | Precoat lbs/ft$^2$ | Body Feed | Flow Rate Range |  | Volume Filtered (gal) | Volume Analyzed (gal) | Cyst Data |  | Recovered/ Applied |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  | gpm | gpm/ft$^2$ |  |  | dosed/gal | Recovered |  |
| 1-D | 0.12 | 0 | 0.10-0.02 | 0.8-0.2 | 1 | 1 | 350,000 | 0 | 0/350,000 |
| 2-D | 0.10 | ? | 1.4-0.6 | ? | 15 | 6 | 6,000 | 3 | 3/36,000 |
| 4-D | 0.10 | 0 | ? | ? | ? | 11 | 10,000 | 1 | 1/110,000 |
| 5-D | 0.15 | 0 | ? | 2.4 | ? | 10 | 6,000 | 0 | 0/60,000 |
| 6-D | 0.36 | ? | ? | ? | 1.35 | 1.35 | 10,000-<br>12,000 | 0 | 0/15,000 |
| 7-D | 0.17 | 170 | 23-11 | 2.6-1.2 | 696 | 17 | 4,500 | 7 | 7/76,500 |
| 8-D | 0.15 | 120 | 24-17 | 3.6-2.6 | 485 | 6 | 3,600 | 1 | 1/21,600 |
| 9-D | 0.15 | 120 | 28-24 | 4.2-3.6 | 648 | 6 | 1,935 | 0 | 0/11,610 |
| 10-D | 0.15 | 0 | 24-14 | 3.6-2.1 | ? | ? | ? | 1 | ? |
| 11-D | 0.15 | 0 | 18-12 | 2.7-1.8 | ? | ? | ? | 0 | ? |
| 12-D | 0.15 | 0 | 25-13 | 3.8-2.0 | ? | ? | ? | 1 | ? |
| 13-D | 0.15 | 0 | 29-18 | 4.4-2.7 | ? | ? | ? | 0 | ? |

Authors' note: Authors of the Army report did not publish complete data on tests 10-D to 13-D. They did make the following comments (pp. 26,27): "*j. Experiments 10-D through 13-D Inclusive. U.S. Army Portable Water Purification Unit, Model 1940.* Converted For Use With Diatomaceous Silica . . .

The purpose of this series of tests was to determine the effectiveness of various grades of diatomaceous silica in the removal of cysts from water. Attention is directed to the absence of cysts in quantity in the effluent from all of the materials tested. Since cysts were removed by all of the materials tested, the significant feature of these tests is that no cysts passed the unit in Experiment 13-D. The filter aid used in the experiment was Johns-Manville Celite 545, which is too coarse for water filtration. Notwithstanding the passage of turbidity in excess of 10 ppm no cysts were found in the effluent samples. Bacterial removal with the several grades of filter-aid tested is shown in Figure 53. It appears, therefore, that any currently manufactured filter-aid in the range of the materials tested will produce satisfactory results."

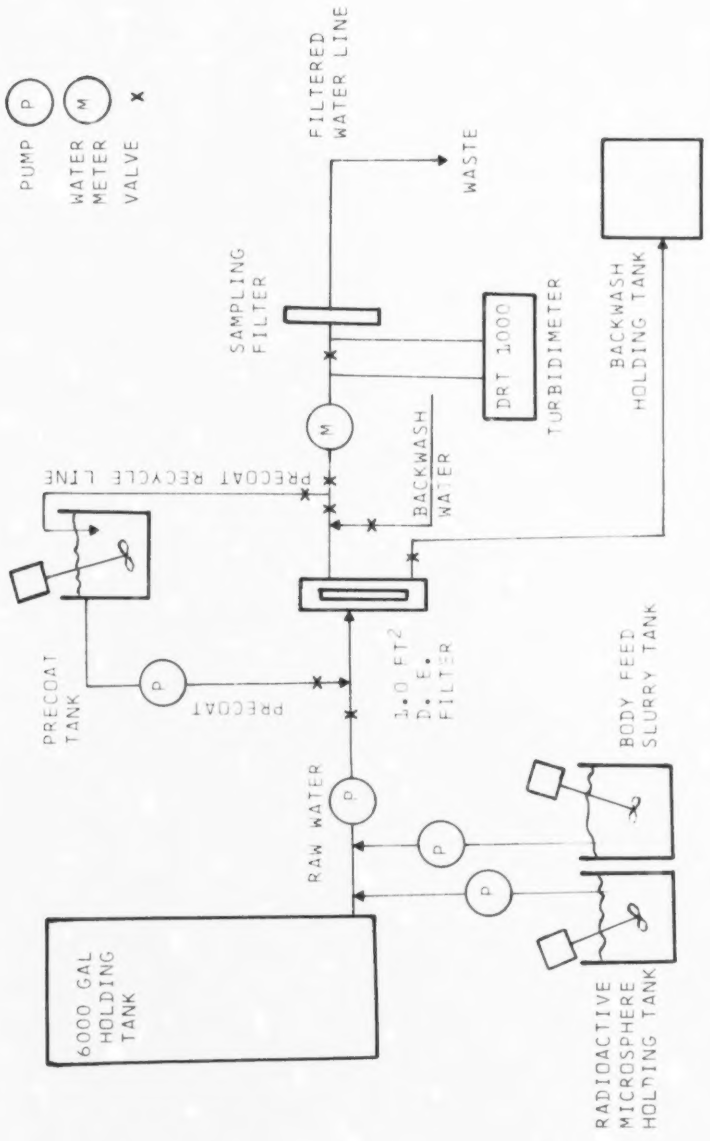
PUMP
P
WATER METER
M
VALVE
x
FILTERED WATER LINE
WASTE
SAMPLING FILTER
DRT 1000
TURBIDIMETER
BACKWASH HOLDING TANK
PRECOAT RECYCLE LINE
BACKWASH WATER
PRECOAT TANK
PRECOAT
1.0 FT²
D. E.
FILTER
BODY FEED SLURRY TANK
RAW WATER
6000 GAL HOLDING TANK
RADIOACTIVE MICROSPHERE HOLDING TANK

**FIG. 1.** *Schematic diagram of diatomaceous earth pilot plant filter system.*

of the filter cake would soon be coated with clay, bacteria, and other particulates found in natural waters and removed by the filter. The pores would be plugged, and a high pressure drop would occur in the filter.

When body feed is added, the filter cake becomes thicker during the run, but because the major constituent of the filter cake is DE rather than removed particulates, the cake is porous, with good flow characteristics. In order to properly adjust body feed dose, some DE filter plant operators add body feed in proportion to raw water particulates. Turbidity is used as an indicator of the particulate content of water, so a body feed-to-turbidity (mg/l to NTU) ratio is sometimes used as a guideline for the needed dose of body feed. An economical body feed-to-turbidity ratio would vary for different raw waters. Body feed-to-turbidity ratios in this work ranged from 2:1 to 35:1. Raw water turbidity was generally 0.6 to 2 NTU.

A DE filter run generally is terminated because of head loss considerations. Because the filter cake increases in thickness during the run, filtered water turbidity should not rapidly increase (turbidity breakthrough) as with granular media filters. Turbidity breakthrough can occur, however, if the flow of water through the filter is interrupted. Interrupting the flow can cause the diatomaceous earth cake to fall off the filter septum. This should not be allowed to occur.

When a DE filter run is completed, the cake is removed from the septum, spent diatomite is discharged, the septum is cleaned, and the filter is prepared for another precoating. Because the spent diatomite in this research contained radioactive particles or *Giardia* cysts, all spent diatomite was saved in a 212 l (55 gal) drum for disposal. The various grades of DE used were commercial products obtained from Johns-Manville (Denver, Colo).

An important feature of the filter system was a membrane sampling filter located downstream from the DE filter. The purpose of this membrane filter was to sample the entire effluent from the test filter and thus attain more reliable data on removal of cyst models. Low turbidity water from an inactive gravel pit was used for these tests.

Detecting and counting *Giardia* cysts in water is difficult, so a model for the cysts was sought. Radioactive microspheres (New England Nuclear, Boston, Mass.), 9 μm in diameter, were used because they were very similar in size to *Giardia* cysts and were easy to trace. The microspheres were resin beads coated with cerium-141 so that the isotope would not leach. The radioactivity of a single microsphere could be differentiated from background in the low level counter used to measure gamma radioactivity on the 5 μm pore diameter membrane sampling filters. Most of the research work utilized the radioactive microspheres.

Two confirmatory tests were performed with *G. lamblia* cysts. A cyst suspension was injected into the raw water ahead of the DE filter. The DE filter effluent was passed through a 7 μm nylon screen (Tabler, Ernst and Traber, Inc., Nitex). After the filter run was completed, the screen was washed repeatedly with 1 to 2 ml of water. The wash water volume was recorded and the water was examined for cysts under the microscope. Cysts were enumerated by the coverslip method and the hemocytometer method.

The efficiency of the effluent sampling method was determined by dosing a quantity of cysts into the effluent line immediately prior to the sampling filter. Water was pumped through the system and the cysts recovered from the sample filter as described. Two trials indicated recovery efficiencies of 8.0 and 7.6%. A factor of 0.08 was used for calculations made to compare numbers of cysts dosed in raw water and cysts recovered from filter effluent.

The removal of cysts by the diatomite filter assembly without filter aid was determined by injecting cysts into the system, pumping water through the system, and enumerating the cysts on the effluent sample filter. A series of 3 tests were conducted. The cyst recoveries were found to be 14.1, 9.0, and 6.1%, averaging 9.7%. Essentially no cyst removal took place, as cyst recovery through the diatomite filter was similar to recovery when cysts were added after the filter.

## Results and Discussion

Test results showed that in most cases a substantial reduction in the concentration of microspheres could be attained. Filter performance was not related to turbidity reduction, a finding very similar to the statement on p. 16 of the Army report. A probable explanation for this is that the cysts were removed even though clays and other particulates that cause turbidity were small enough to pass through the filter. The smallest cyst dimension would be about 7 $\mu$m, whereas clays and bacteria would be as small as 1 $\mu$m, and thus more likely to pass through the pores of the filter cake. Therefore, performance is related to good operating procedures and not necessarily to turbidity removal.

Data obtained from the filter runs in which microspheres were used are plotted to show the differences in filter performance that are related to filter operating procedures. Specifically, reduction of microsphere concentration met or exceeded 99.9% in 21 tests when the diatomite precoat applied was at least 91g/0.1m$^2$ (0.2 lb/ft$^2$) of filter surface, and when body feed was utilized. In 2 tests with these operating conditions, reduction of the microsphere concentration was less than 99.9%.

Filter operation with a precoat thickness of less than 91g/0.1m$^2$ (0.2 lb/ft$^2$) (2 runs), or with no body feed (3 runs), or with less than 91g/0.1m$^2$ (0.2 lb/ft$^2$) of precoat as well as no body feed (3 runs) resulted in removal of less than 99.9% of the microspheres. The test results show the importance of properly establishing the filter cake by using an adequate amount of precoat diatomite, and of maintaining the integrity of the cake during a filter run by applying body feed.

Data indicated that filter efficiency was related to precoat thickness. Therefore a series of tests was set up to evaluate this. The filter was operated with precoat only. The tests were done with a single batch of water so that turbidity differences would not influence the results, shown in Fig. 3. Efficiency of microsphere removal increased with increasing precoat

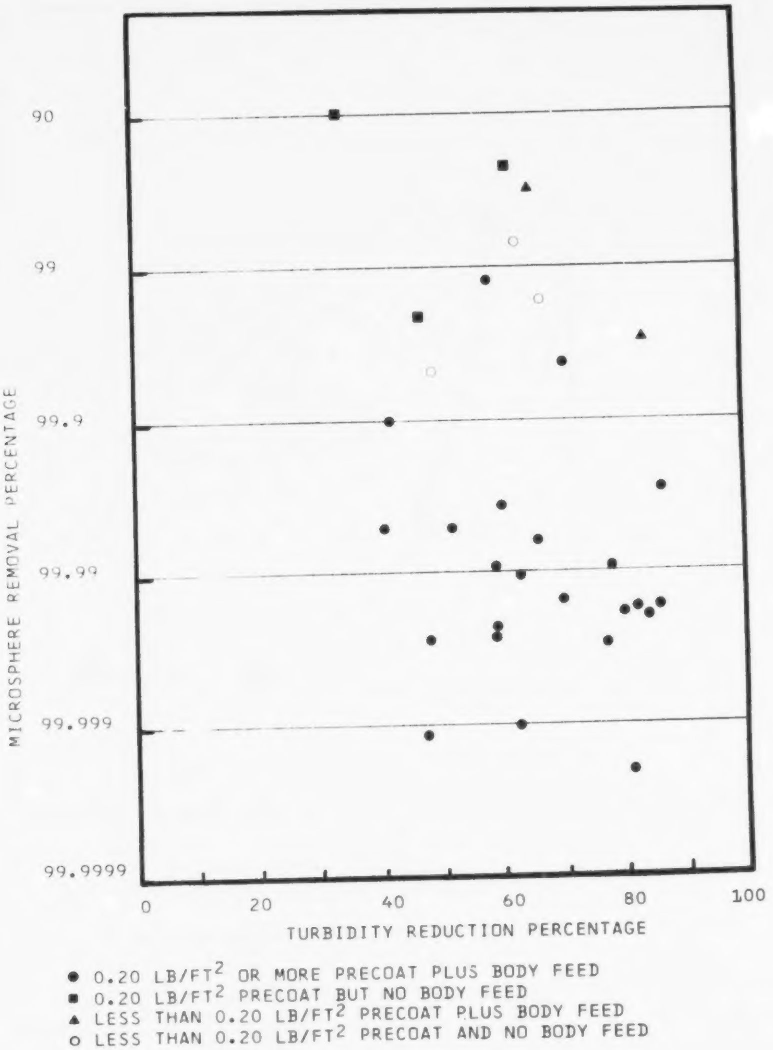

FIG. 2. *Relationship of water quality and filter operation.*

thickness up to 91g/0.1m$^2$ (0.2 lb/ft$^2$). Additional precoat did not improve filter performance.

Results from the membrane sampling filters showed that the microsphere removal capability of the DE filter usually improved during the run. Ordinarily during a filter run the sampling filter was changed once. Counting data showed that the relative number of radioactive beads caught during the first portion of the run nearly always exceeded the proportion of

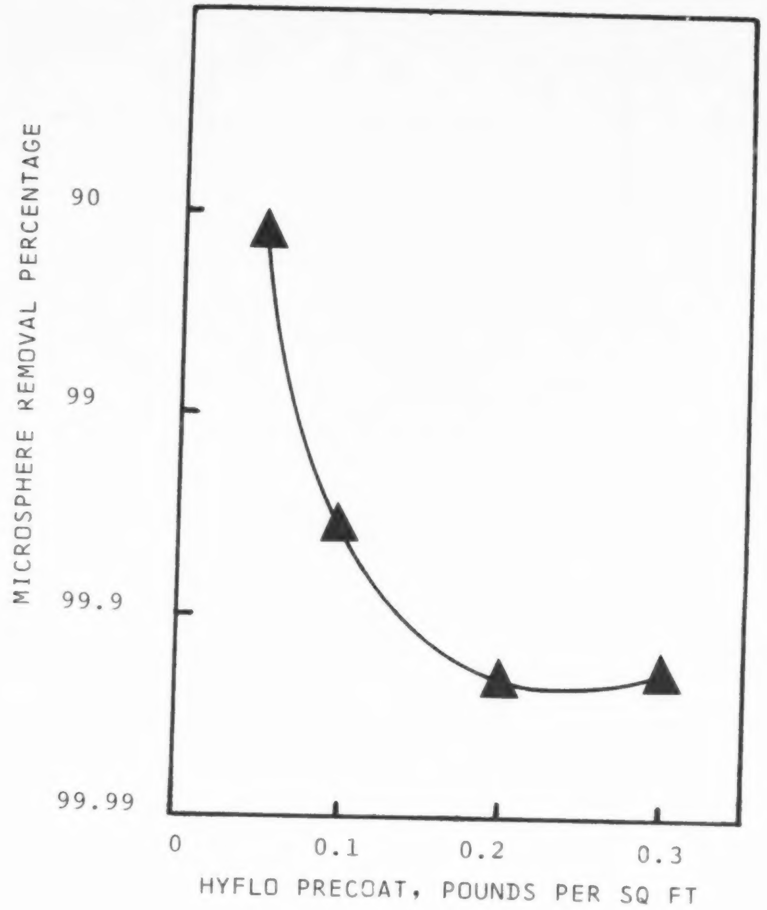

FIG. 3. *Effect of precoat amount on microsphere removal.*

the DE effluent that had passed through the filter (Table 4). Conversely, the fraction of beads caught on the filter for the latter part of the run was nearly always less than the proportion of effluent passing the sample filter. These data suggested that addition of body feed and removal of particulates produce a filter cake that is progressively more effective for particulate removal as the filter run continues.

Filtration research involving actual *Giardia* cysts was carried out to verify the results with the microsphere studies. The *Giardia* cysts were dosed into the diatomite filter influent and filtered through a very coarse filter aid without the addition of body feed because these were the most inefficient conditions for particle removal as indicated by filtration runs involving the microspheres. The cysts were dosed immediately upon run initiation and the

*Table 4. Effect of run length on microsphere removal*

| Run No | Effluent Sample No | Precoat & Amount | % of Total Volume of Filtrate Samples and % of Microspheres Expected | % of Total Microspheres Recovered in Effluent Sample | Ratio of Recovered To Expected |
|---|---|---|---|---|---|
| 28 | 1 | JM 545/JM512 | 62 | 63 | 1.02 |
| | 2 | 0.1 lb/ft² ea. | 38 | 37 | 0.97 |
| 29 | 1 | JM 545/JM 512 | 73 | 88 | 1.20 |
| | 2 | 0.1 lb/ft² ea. | 27 | 22 | 0.81 |
| 34 | 1 | JM 545/HYFLO | 65 | 0.1 | .001 |
| | 2 | 0.1 lb/ft² ea. | 35 | 99.9 | 2.86 |
| 58 | 1 | HYFLO | 21 | 59 | 2.81 |
| | 2 | 0.2 lb/ft² | 31 | 30 | 0.97 |
| | 3 | — | 48 | 11 | 0.23 |
| 59 | 1 | HYFLO | 23 | 42 | 1.83 |
| | 2 | 0.05 lb/ft² | 77 | 58 | 0.75 |
| 37 | 1 | JM 503 | 77 | 28 | 0.36 |
| | 2 | 0.10 lb/ft² | 23 | 72 | 3.13 |
| 57 | 1 | JM 503 | 53 | 80 | 1.51 |
| | 2 | 0.10 lb/ft² | 47 | 20 | 0.43 |
| 56 | 1 | JM 503 | 58 | 79 | 1.36 |
| | 2 | 0.10 lb/ft² | 42 | 21 | 0.50 |
| 55 | 1 | JM 535 | 23 | 97.7 | 4.26 |
| | 2 | 0.10 lb/ft² | 29 | 0.6 | .02 |
| | 3 | — | 48 | 1.7 | .04 |
| 43 | 1 | JM 560 | 60 | 72 | 1.2 |
| | 2 | 0.20 lb/ft² | 40 | 28 | 0.7 |

run continued for a sufficient time to overcome the detention time of the filter unit and piping, approximately 30 min.

One run utilized 91g/0.1m² (0.2 lb/ft²) precoat of JM 560 with $2.2 \times 10^6$ cysts dosed. Ten percent of the volume of the sample screen washing water was examined. No cysts were detected. With corrections of recovery efficiency (0.08) and volume sampled (0.10), if all cysts dosed had passed through the DE filter, 18,000 should have been counted. A reduction of cysts to less than one per 18,000 initially present is indicated. In a second run with 45g/0.1m² (0.1 lb/ft²) of JM 545 as a precoat, $1.3 \times 10^7$ cysts were dosed but again none were observed in the 15% of the filter washings examined. Applying the factors (0.08 x 0.15), $1.6 \times 10^5$ cysts should have been observed if all passed the DE filter and 8% were recovered from the sample filter. The filter reduced the cyst concentration to less than 1 per 160,000 initially present.

If a diatomaceous earth filter was as efficient for *Giardia* cyst removal as it was for microsphere removal, a very small percentage of cysts would pass through the filter. Failure to observe cysts in the effluent suggests that cysts are more easily removed than microspheres or that the cysts in the effluent were not present in a large enough number to be detected by the sampling methods employed in this study, therefore the use of the microsphere model is conservative.

### Conclusions from DE Tests

DE filtration for *Giardia* cyst removal is practical and effective provided these filters are properly maintained and operated, both essential factors.

Thickness of DE precoat had a greater effect on microsphere removal than did DE grade (particle size).

Precoat thickness for DE filters operated for *Giardia* cyst removal should be at least 91g/0.1m$^2$ (0.2 lb/ft$^2$) to provide an effective barrier to cyst passage.

DE filter efficiency for microsphere removal generally increased as the filter runs progressed.

DE filters used in the production of potable water should not be operated without body feed addition.

Measurement of effluent turbidities is an inadequate measure of the DE filter's performance in regard to *Giardia* cyst removal so reliance must instead be placed on use of proper treatment techniques with an adequate amount of precoat and body feed diatomaceous earth.

## Granular Media Filtration Studies

A program of laboratory and pilot plant research on *Giardia* cyst removal by granular media filtration is now underway in the Drinking Water Research Division. Preliminary jar test experiments in the laboratory are designed to yield information on alum and polymer doses that will effect reduction of *Giardia* cysts in granular media pilot plant filter tests.

The source of *G. lamblia* cysts for the first series of tests was an infected human donor. After a number of apparently successful jar tests had shown that coagulation, flocculation and sedimentation had reduced the cyst concentration by 99%, the cysts were shown to be nonviable. Work with these cysts was discontinued because of concern that test results with nonviable cysts might not be representative of results that would be obtained with viable cysts.

The Health Effects Research Laboratory has obtained mice infected with viable *Giardia muris* cysts. The work is now being performed with *G. muris* cysts. Preliminary results look promising. Analysis of a sample of *G. muris* cysts in distilled water at pH 5.5 showed that the zeta potential (related to electrophoretic mobility) was -26 millivolts with a standard deviation of 2.8 millivolts. Initial tests of flocculation and sedimentation also suggest that *G. muris* cysts can be readily removed from water. If so, granular media filtration also is likely to be successful. This will be studied in the pilot plant.

## FUTURE RESEARCH

The Drinking Water Research Division has funded a study of water treatment for *Giardia* cyst removal with research to be done by the

Department of Environmental Health at the University of Washington. The objective of this study is to develop information on the removal of *Giardia* cysts from water by filtration and associated treatment processes such as coagulation, flocculation, and sedimentation. Laboratory studies will be conducted during the first year of the grant to obtain information on treatment techniques that can remove the cysts from water. This information will provide direction for field studies to be conducted in the second year of research. Field studies will be done using pilot plants, full scale facilities, or both. Granular media filtration and diatomaceous earth filtration will be studied. The Department of Social and Health Services, State of Washington, will cooperate in the field studies. This project, already under way, is expected to continue into 1980. EPA studies at the Environmental Research Center will complement the university research.

## SUMMARY

Granular media filtration has been shown to be effective for removal of *E. histolytica* cysts from water. In the 1930's Baylis reported removals exceeding 99.99% when coagulated water was filtered through 61 cm (24 in) of 0.5 mm effective size sand at 7.7 lpm/0.1m$^2$ (2 gpm/ft$^2$). U. S. Army studies during WW II showed that removals exceeded 99.9% when coagulation, flocculation and sedimentation preceded filtration at 24.4 lpm/0.1m$^2$ (6.35 gpm/ft$^2$) through 46 cm (18 in) of 0.36 mm effective size sand. A preliminary measurement of the zeta potential of *Giardia muris* cysts showed that these cysts are electronegative, as are most clays and bacteria, so granular media filtration should remove *Giardia* cysts. Proper operating procedures must be used at granular media filtration plants. Army data showed that many more cysts passed through the sand filter when good coagulation was not employed.

Diatomaceous earth filtration was shown by the US Army to effectively remove *E. histolytica* cysts. Preliminary experiments with *G. lamblia* cysts recently indicated DE filtration was just as effective for these cysts as for *E. histolytica.* Research with radioactive microspheres showed a relationship between filter operating procedures and effluent quality. To assure the most effective filter performance for removal of cyst-sized particles, plant operators should apply 0.2 pounds of precoat diatomite per square foot of filter surface. Body feed should always be used.

Both granular media and diatomaceous earth filtration plants should be able to remove cysts. Experimental data show that use of proper operating techniques is essential if these plants are to perform effectively.

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## Discussion

E. LIPPY: Would you comment on the use of a DE filter as a sampling unit for *Giardia* detection?

G. LOGSDON: I think it would be possible to do that if you could separate the diatomaceous earth from the *Giardia* after the water had passed through the DE filter. The Army work with amebic cysts and the DE filter showed that the Army obtained practically complete removal of the cysts. A number of those filter runs had no cysts detected in the effluent. I do not know, however, how you would separate diatomite from cysts in order to use this as a detection technique.

D. JURANEK: At the time that CDC was developing the sand filter, diatomaceous earth was used as a first coat medium because that is what came with the swimming pool filter that was used. You pointed out exactly the problem; there is no good way to separate *Giardia* cysts from the diatomaceous earth.

B. LIECHTY: I think it is really encouraging to see a research person associated with EPA recognize the importance of quality plant operations. I think there is a lesson to be learned from the waste water treatment program where literally millions of dollars were spent to design and construct treatment facilities that were very effective, on paper. Experience is now showing that, in some cases, effluent standards are not being achieved, often as the result of improper operation of the plants.

# SESSION VI - OTHER RECENT RESEARCH (ABSTRACTS)

*Chairman - Walter Jakubowski,*
*U.S. EPA, HERL, Cincinnati, Ohio*

## In Vitro Model for Analyzing the Attachment/ Release of Trophozoites of *Giardia:* Evidence for the Involvement of Contractile Proteins and the Effect of Atabrine

**L. S. Erlandsen, D. E. Feely, J. V. Schollmeyer, D. G. Chase**

## Report of Endosymbiosis in *Giardia muris* and Comparison of Organelle Distribution in *Giardia muris* and *Giardia lamblia*

**P. Nemanic, R. L. Owen, D. P. Stevens, J. C. Mueller**

## Intestinal Distribution, Attachment and Relationship of Giardial Trophozoites to Peyer's Patches During Acute Infection in the Immunologically Intact Host

**R. L. Owen, P. Nemanic, D. P. Stevens**

## Giardiasis in Vail, Colorado

**W. Jones and T. Edell**

## The Presence of *Giardia lamblia* Cysts in Sewage Sludges from the Chicago Area

**J. C. Fox, P. R. Fitzgerald**

## Axenically Cultivated *Giardia lamblia:* Growth, Attachment, and the Role of L-Cysteine

**F. D. Gillin, L. S. Diamond**

# In Vitro Model for Analyzing the Attachment/Release of Trophozoites of *Giardia:* Evidence for the Involvement of Contractile Proteins and the Effect of Atabrine

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The direct interaction and/or attachment of the trophozoites of *Giardia* with the microvillous border of the intestinal epithelium appears to be a common factor in postulated mechanisms for the pathogenesis of this intestinal protozoan. Using a method for the isolation of trophozoites of *Giardia muris* developed in our laboratory (Feeley and Erlandsen, 1978, Anat. Rec. 190:393) the mechanism of attachment of trophozoites of *Giardia* to surfaces in vitro has been analyzed by means of: 1) morphological examination including scanning, transmission, and high voltage electron microscopy, 2) functional studies using light microscopy cinematography with phase and Nomarski interference optics, 3) immuno-biochemical studies on the presence and distribution of contractile proteins in the trophozoite, and 4) the effect of various drugs including Atabrine on the attachment and/or release of trophozoites from surfaces. High voltage and transmission electron microscopy have revealed the presence of fibrous masses in the edge of the adhesive disc (lateral ridge), in rod-like masses paralleling intracellular axonemes of the antero- and postero-lateral flagella, and fibrous masses associated with the axostyle paralleling the caudal flagella. Using SDS-gel electrophoresis, co-migrating bands similar to avian contractile proteins have been identified for myosin, $\alpha$ -actinin, tubulin, and actin. Immuno-fluorescent studies using antisera to avian $\alpha$ -actinin and actin have demonstrated $\alpha$ -actinin localization in the lateral ridge of the adhesive disc and in the rod-like fibrous masses associated with the postero-lateral flagella. Actin localization was also seen in these same fibrous masses as well as in the median body, an organelle composed of microtubules. Cinematography of attached trophozoites revealed that the tail of the trophozoite is capable of vigorous lateral movement and that dorsal flexion of the tail occurs just prior to detachment from a surface. Collectively, these observations suggest that the rim of the adhesive disc (lateral ridge) may possess contractile properties which govern shape changes in the adhesive disc. Attachment of the disc to the surface followed by a reduction in the size of the disc due to contraction of the rim would create a negative pressure to maintain attachment. Relaxation of the contractile elements in the rim presumably during or prior to dorsal flexion of the tail of the trophozoite would vent the negative pressure and permit detachment from the surface. Studies on the effect of Atabrine on trophozoite attachment revealed that 1 μg/ml of Atabrine (quinicrine-HCl) produced 22% detachment of trophozoites in 10 min whereas 10 and 100 μg/ml produced 45 and 92% detachment respectively. After 30 minutes of exposure, 44% detachment was observed at a dosage of 1 μg/ml while 85 and 99% detachment were observed at 10 and 100 μg/ml of Atabrine. The exact antiparasitic mechanism of Atabrine is not clear although it is known to inhibit nucleic acid synthesis, however, these data clearly demonstrate that it may act directly on the attachment of trophozoites to surfaces through an as yet undefined mechanism. (Supported in part by USPHS Grant No. AM 18344)

## Discussion

B. WHEAT: Those were beautiful micrographs. What type of fixation, dehydration, et cetera, did you use for scanning preparation?

S. ERLANDSEN: We use a variety of fixatives, mainly glutaraldehyde type fixatives. Sometimes we have osmium associated with them in what we call a cocktail fixation. It is not a good name for it, but they are actually in the same vial together. These specimens are all critical-point dried. We have not looked at *Giardia* with a freeze-drying technique.

D. STEVENS: My compliments also on your usual elegant presentation. Do you have any thoughts on what the discriminatory character of trophozoites is for surfaces, or do you think it is just a rather mindless operation on the part of the trophozoite? I realize this goes beyond your data, but I wondered if you had reached any conclusions. In the sense that we see attachment in the intestine, in particular areas and not in others, is there something in terms of proteins or pH?

S. ERLANDSEN: There is no answer at this particular point. We are working with in vitro systems that will allow us to hopefully pinpoint some of the answers that you would like to have on that subject. Perhaps the more interesting question would be in relationship to something that E. Meyer raised a little earlier, that *Giardia* may have an affinity for heat. The fact that this organism likes warm surfaces such as epithelium might help to attract it, and to counteract this one person even suggested that perhaps we should drink a lot of ice water. A lot of people like to chew cracked ice, but in Mexico that is not a very good thing to do because of other things in the water.

D. STEVENS: How did you test the viability of the trophozoites?

S. ERLANDSEN: We tested the viability in four ways. First of all we looked at dye exclusion using either trypan blue or erythrosin B. We have done some labelling with isotopes and have looked at spontaneous release over time. We have correlated each of these tests also with trophozoite motility. Through all of these experiments we are able to keep viability at 90% up through about 180 min so we feel that for the very short acute experiments that we are dealing with, viability is not a factor. All of our work is usually done within a half hour to an hour at the most.

T. NASH: I noticed in the micrographs that there was nothing attached to the organisms. Is this a common occurrence? Do you ever see anything attached?

S. ERLANDSEN: In vivo we have seen things that look like bacteria associated with them, but we have not seen those other than during early stages of the isolation procedure. It appears to be an artifactual association with the trophozoite flagella. In the second cycle during isolation, no microorganisms are seen attached to *Giardia* , and if you go through a third and a fourth cycle there are few if any microorganisms present.

T. NASH: I was particularly interested in the bacteria. I am interested in any mucus, or white cells.

S. ERLANDSEN: No. I am looking forward to hearing Dr. Owen's paper on that.

# Report of Endosymbiosis in *Giardia muris* and Comparison of Organelle Distribution in *Giardia muris* and *Giardia lamblia*

**Paulina Nemanic, Robert L. Owen, John C. Mueller,**
**and David P. Stevens**

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Ultrastructural observations of *Giardia muris* in the mouse model system revealed endosymbiotic microbes not previously reported in *Giardia*. Endosymbionts were 240 to 260 nm wide and 600 to 1400 nm long with internal structure consistent with bacteria. Endosymbionts were not seen entering *Giardia* but appeared to be passed on during giardial replication. They were observed to divide within the cytoplasm of *Giardia* without evidence of digestion by the host in either trophozoites or cysts and were found in groups of mice serially infected with cysts passaged repeatedly through CF1 mice. Phagocytosis of particles as large as endosymbionts was not seen. Instead, a number of previously undescribed organelle features point to a feeding mechanism for *Giardia* by means of small (40 nm) micropinocytotic vesicles located immediately beneath the dorsal and exposed ventral surfaces of the trophozoite. Vesicles fuse with larger peripheral tubules found in the same area and previously presumed to be the primary feeding vacuoles. Well-developed RER, previously unreported in *Giardia*, was observed and confluence of RER cisternae with these tubules is suggested as the source of digestive enzymes, with discharge of unusable products via larger exocytotic vesicles along the dorsal surface of the organism. Endosymbionts were concentrated centrally in the nuclear area and were uncommon in the peripheral feeding regions. In comparison with *G. lamblia* the same organelles as in *G. muris* were found but not the endosymbionts. Replication and transmission of foreign DNA by endosymbionts within *Giardia* strains must be investigated to determine whether they alter trophozoite pathogenicity, metabolism, range of infectivity, antigenic surface characteristics or host specificity as endosymbionts have been shown to do in other protozoa.

(*Editor's note:* For the complete paper on this topic see Nemanic, P. C., R. L. Owen, D. P. Stevens, and J. C. Mueller. 1979. Ultrastructural observations on giardiasis in a murine model. II. Endosymbiosis and organelle distribution in *Giardia muris* and comparison with *Giardia lamblia*. Jour. Inf. Dis. August 1979, *In Press*)

## Discussion

B. WHEAT: There is a technique that has been used with coccidia in testing whether sporozoites actually penetrate cells by using ruthenium red, and it stains very darkly and does not enter cells that are attached. You might try putting that in your suspension and seeing if that helps.

P. NEMANIC: I will try it.

S. ERLANDSEN: Do you see any difference in the amount of RER associated where these symbionts are present in the organism?

P. NEMANIC: Since all had endosymbionts, I cannot say.

S. ERLANDSEN: When you showed us the long tubules, there were median bodies that had short dorsal ribbons extending up from them, and it looked like the median bodies were dissolving; there was much more space between the microtubules than one would expect to see in a microtubule organelle like that. I wonder if that is not perhaps the beginning stage of encystment and what you are seeing is very similar to what H. Sheffield was describing with the long tubules. Perhaps this is the release of the cyst wall itself through exocytosis of that material.

P. NEMANIC: That is possible. All of this was done in vivo, in the animal; and we have some micrographs which show trophozoites next to cysts, and whenever we find that, the cytoplasm nearest the dorsal edge is very, very light and the trophozoite itself looks very condensed.

S. ERLANDSEN: Endosymbionts in other intestinal protozoa are common. As far back as the turn of the century Dr. Dobell made a very beautiful drawing of endosymbionts present in some of those protozoa, including *Hexamita intestinalis*. The RER tends to be arrayed in fairly substantial amounts around these endosymbionts, and it is very interesting to note that these areas were referred to as higher metabolic centers within the protozoans.

D. PRICE: Recently, I have noted a number of what appear to be ingested bacteria in *Giardia* trophozoites that I see in light microscopy, and also I see what appears to be some digestion of these organisms since they vary in size from the time they enter until the time they seem to be extruded, and I wonder what evidence you have to show that this is not a vacuole in which the organism has been ingested, or whether it is an actual compartment in which the organism is living.

P. NEMANIC: We never find any evidence of digested endosymbionts. We have seen some evidence of what looks like division taking place within the endosymbionts.

D. PRICE: All the other intestinal protozoa do ingest bacteria. Is this the one exception?

P. NEMANIC: We have never seen any evidence that *Giardia* are ingesting them, and one would not expect to find too many bacteria floating around the upper duodenum.

S. ERLANDSEN: I think in some intestinal protozoa they are not always ingested. If you consider the order *Hypermastigida*, there are endosymbionts that metabolize cellulose, and you have a number of cases like that where the organisms exist either in or on the host itself. Perhaps you would comment on the trophozoite you saw that had 35 endosymbionts within a single section. Did it look viable?

P. NEMANIC: It looked wonderfully viable. It looked just like all the rest, only freckled.

A. TOMBES: Following along with that question about the digestion, did you see any lytic activity?

P. NEMANIC: I have not seen anything that looks like a lysosome. I expect that the peripheral tubules may be a heterogeneous group, that is, they all look alike, but we are doing histochemical testing, and we may find that perhaps some of these are digestive organelles. I have looked at literally thousands of *Giardia* and have never once seen any evidence of ingestion of bacteria. I have only seen a few invaginations filled with material that is always different from the debris floating near the trophozoites.

# Intestinal Distribution, Attachment and Relationship of Giardial Trophozoites to Peyer's Patches During Acute Infection in the Immunologically Intact Host

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Increased severity and incidence of symptoms of giardiasis in immunodeficient humans and laboratory animals points to an abnormal immune response to *Giardia*. We studied prospectively infected healthy mice in a well-described model system, using ultrastructural techniques to establish normal distribution of trophozoites, their relationships to intestinal mucosa, particularly Peyer's patches and cecal lymphoid patches, and structural indications of the normal reaction of intestine and intestinal immune organs to *Giardia muris*.

We found that trophozoites which colonize only the proximal 25% of the intestine adhere to microvilli of columnar cells near the bases of villi, wedge into furrows in the epithelial surface, or lodge in mucus within the unstirred layer. Density of trophozoites colonizing the jejunal epithelium correlated with cyst excretion numbers prior to as well as at peak cyst shedding. *Giardia* do not adhere to Peyer's patch M cells, which transport small particulate material from the lumen to the lymphoid system, but appear to enter intestinal lymphoid structures by incursions through defects in the lymphoid follicle epithelial barrier. Lymphocytes leave the epithelium and attach to *Giardia* and *Hexamita* in the lumen. We conclude that *Giardia* colonize only the proximal 25% of the small intestine and maintain this location by adhesion and localization in the unstirred layer. They produce no apparent ultrastructural damage in normal mice but elicit a previously undescribed intraluminal cellular immune response prior to clearance by the host.

(*Editor's note:* For the complete paper on this topic see Owen, R. L., P. C. Nemanic, and D. P. Stevens. 1979. Ultrastructural observations on giardiasis in a murine model. I. Intestinal distribution, attachment and relationship to the immune system of *Giardia muris*. Gastroenterology 76:757-769.)

## Discussion

W. JAKUBOWSKI: I would like to compliment you and Dr. Nemanic and Dr. Erlandsen on some very fine electronmicroscopic work, but I must admit that I feel somewhat like an adolescent getting his first look at Penthouse Magazine — I am fascinated by what I see, but I am not sure I understand it. Based on your observations, would you or any of the others care to postulate a mechanism for the production of symptoms in giardiasis?

R. OWEN: Well, we do a lot of fantasizing about this, as with Penthouse Magazine, I suppose. You begin to feel a very intimate sort of relation with *Giardia*, like thinking about taking them for walks and things of that nature.

When there is co-infection with a virus or something else that causes loss of integrity of the barrier along the wall of the intestine, more of the *Giardia* may wander into the tissue. In a sensitive host capable of a cellular immune response, one can anticipate that there would be an inflammatory response within the tissue which might disrupt or damage the villi of the intestine.

This is a question that we have dealt with tangentially in the last couple of days: why do some people get sick and not others? Why can someone carry *Giardia* for a long time and not have symptoms and then begin to have symptoms? Dr. Wolfe suggested that perhaps there may be a co-infection with some other agent so that what we see when there is symptomatic giardiasis may be a summation effect. The *Giardia* are minding their own business, eating a little bit of your lunch, and along comes some other factor - infection, stress, irradiation or something else - and they wander into the epithelium. Then there is a cellular immune response within the gut wall that may disrupt the epithelium and create a change in the turnover rate so that less mature epithelial cells are migrating up out of the crypts and covering the surface of the villi, cells that are less developed and less able to absorb and transport nutrients from the lumen of the host.

S. ERLANDSEN: Your results with the mouse seem to be quite different from what has been reported to occur in the rat in terms of distribution. In the studies that have been done (Hegner, R. and L. Eskridge, J. Protozool, 24:511, 1938) where they were sacrificing 20 to 50 animals per group, it was found that a bimodal distribution of *Giardia* existed in the rat intestine, trophozoites were not present in the first 30 to 40 cm of the loop of the duodenum and jejunum, indicating that there was something in the pancreas affecting them, and I think you are stating the opposite of that. It was also shown that there is one high incidence of colonization in the jejunum and another one down in the ileum as well. Do Dr. Stevens or you ever see variations of this distribution? Do you ever find them lower, or have you looked at a large enough series of animals to be sure that they do not get down into other areas as well?

R. OWEN: With electron microscopy 10 animals can seem like a very large series by the time you have gone through all the sectioning and preparation. There are some studies on distribution that indicate that when you change the diet one can change the distribution. In puppies, for instance, you can move them up and down in the intestine by changing the diet, and by changing the diet I presume what one changes is motility. When there is an overgrowth or a massive infection, you find them everywhere: in the gallbladder, the colon, the stomach. It may have to do with the numbers of organisms that are present and the resistance of the host. We did not fast these animals because we did not want to stress them. If the animals were fasted, we might see something different, and obviously in other animals other things have been seen.

One thing that we speculated about is that there might be some sort of membrane configuration over the microvilli that could induce attachment, that somehow *Giardia* might read the surface to get a clue that they were in the upper jejunum. Dr. Marilyn Etzler (Etzler, M. E. and Branstrator, M. L., Jour. Cell Biology, 62:329, 1974) at Davis has used lectins with fluorescent labels and found that there are proximal intestinal membrane configurations at the bottom of the villus and not at the top, and they coincidentally happen to be just in the areas where the *Giardia* seem to hang on. Whether the *Giardia* have evolutionarily clung to this sort of membrane as an indication of where they should attach we do not know, but this is what we have seen.

S. ERLANDSEN: In your micrographs you had a radial spoke arrangement on the adhesive disk. It looked like areas of elevations and depressions, and that puzzles me greatly. Do you have any structural correlation for this, or do you think there is some other explanation, perhaps?

R. OWEN: I do not know.

D. STEVENS: In response to Dr. Erlandsen's earlier question about looking at more animals for evidence of infection in the upper part of the small bowel, when we first started working with a model 4 years ago, Dr. Roberts-Thomson working in my lab, made the observations of those villus:crypt ratios which we talked about yesterday, but I was not terribly enthusiastic about their control. It disheartened me a bit that he only saw the changes in the first 10 to 15 cm of the small intestine. The reduction in the villus:crypt ratio did not occur beyond that point, so that all observations thereafter were made arbitrarily at 10 cm. In retrospect, these observations are probably valid and may be related to interaction with the adhesive trophozoites.

S. ERLANDSEN: If one measures villus to crypt height, one has to be very careful of liver and pancreatic function because it has been shown by transposition of the intestine that the height of the villus is dependent upon pancreatic and bile flow.

G. JACKSON: Just as an aside, with the intestinal nematodes one often sees whole coats of host cells, both polymorphonuclear and monomorphonuclear white cells, when the nematode projects into the lumen and there is a host reaction.

# Giardiasis in Vail, Colorado

**Wesley E. Jones and Timm A. Edell**

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Within a 2-day period in April 1978, 37 cases of laboratory-confirmed giardiasis from Colorado and other states were reported to the Parasitic Diseases Division, Center for Disease Control. All were in residents of or visitors to Vail, Colorado, during March or April 1978.

An epidemiologic investigation was initiated consisting of a questionnaire and a stool survey of residents of the city and an adjacent control town. Information was obtained on 741 residents of Vail; 429 (58%) gave a history of diarrheal illness that occurred February, March, or April 1978. The epidemic curve demonstrated a rise in the number of acute diarrheal illnesses beginning on March 14-16 and reaching a peak on April 1-2. No statistically significant differences in illness rates were noted by age, sex, or length of residence. However, there were significant differences between those who drank no water and those who drank 1 or more glasses per day ($P<0.05$). None of the 7 separate Vail Valley water districts could be statistically implicated as the source. Seventy-two stool examinations revealed 12 cases (17%) of *Giardia lamblia* infection. None of 37 specimens from the control town were positive ($P<0.05$). This outbreak emphasizes the continuing need for investigative work into the epidemiology and pathophysiology of *G. lamblia* infection.

# The Presence of *Giardia lamblia* Cysts in Sewage and Sewage Sludges from the Chicago Area

**J. Carl Fox and Paul R. Fitzgerald**

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Raw sewage and anaerobically digested sludge collected from 5 sewage treatment plants operated by the Metropolitan Sanitary District of Greater Chicago were examined biweekly (February 1976 to May 1977) for the presence of parasite ova or cysts. Eleven genera of parasites were found: *Ascaris* sp., *Toxocara* sp., *Toxascaris leonina, Trichuris* sp., *Enterobius vermicularis, Taenia* sp., *Hymenolepis* sp., *Eimeria* sp., *Isospora* sp., *Entamoeba coli* and *Giardia* sp. *Giardia* cysts were never observed by sucrose flotation, however, they could be detected and counted by examination of stained wet mounts. Buffalo black, methylene blue and phyloxine B were effective stains for use with wet mounts. The *Giardia* cyst counts ranged from 333 to 2000/gallon in some raw sewage samples. In most cases there were too few to count. Cysts were not detected in anaerobically digested sludge, even though the organic solids were much more concentrated. This indicates that these cysts may not survive the anaerobic digestion process.

*Giardia* cysts were most prevalent in sewage composed primarily of domestic waste. Cysts were seldom found in sewage from industrial areas. Cysts were most often encountered during the warm months (April - September) but were occasionally present in raw sewage at other times. The actual incidence of *Giardia* in sewage may be higher, as *Giardia* cysts are difficult to detect when present in small numbers. Improved methods are needed to detect these parasites in sewage.

## Discussion

R. OWEN: Were these separate or combined storm and sanitary sewers that you examined? Could the presence of dogs have contributed to your findings?

J. FOX: It was all one system and dogs could have contributed to our findings. If you walk down the streets of Chicago you have to tiptoe at times since there is a large amount of dog feces on the streets which can be washed into the sewers by rain.

R. OWEN: Is there any suggestion from anything you have that some of those cysts you found in the two-week digested sludge were viable?

J. FOX: I do not know whether they were viable. I was looking at formalin treated specimens. They looked just like the cysts that I found in the raw sewage, but that was only at one plant, and that plant often had problems with their digesters. They have shut them down sometimes and used them just as holding tanks. I am sure that at times they gave samples that were not representative of what they should have been giving me, which was the digester drawoff that should have been two weeks old. I may have received some new sewage. Also, digesters are charged every day. These digesters hold three and a half million gallons. They put in one-fourteenth of that volume each day and they draw out that much. I may have just received a fresh specimen.

R. CYPESS: To indirectly answer Dr. Owen's question, serum samples taken from swine that were fed sewage were analyzed in my laboratory for the presence of *Toxocara* antibody. These animals developed an antibody response suggesting that the embryonated eggs were definitely viable. There is a difference in resistance of *Toxocara* and *Giardia*, of course, but there is an indication that *Toxocara* can survive and remain viable in sewage.

J. FOX: The second slide I showed you, the *Toxocara* slide, was an egg recovered from a holding basin in Fulton County, and that egg had already been in that holding basin for two years in an unembryonated state. It was removed and embryonated in the laboratory. It was there that long because the sanitary district stopped shipping sewage down the river for awhile, so there was that time period when they were not hauling anything there. That sample was collected during that time and indicates that these eggs can remain viable for a long time in an unembryonated state.

D. PRICE: If you fix *Giardia* in neutral formalin, they will last indefinitely. I am sure CDC is still sending out material collected from honey pots in Japan after World War II, so I think that they will stay in good shape for a fairly long time in formalin.

J. FOX: I do not think *Giardia* is a problem in sewage sludges, but I do think there is a possible problem with ascarids — probably *Ascaris lumbricoides*. Cook County has one slaughter house which slaughters swine, but that one slaughter house does not dump sewage into all the plants that I was investigating, and I found the eggs at all the plants. I think there are human factors contributing to the *Ascaris* eggs that we are seeing. These eggs survived that long period in the holding basins, and they would embryonate after we brought them back into the laboratory. We seldom found an embryonated egg right out of the basin.

W. JAKUBOWSKI: Would you care to speculate on the possibility of examining sewage as a means of conducting surveys on prevalence of *Giardia* in populations?

J. FOX: I think there is a very good possibility of using sewage plants. I believe it is a valid approach to determine whether the cysts are present in the community. At least then you know that there is a host somewhere harboring *Giardia*. You can then pick out those communities and key in on them and look for the source of *Giardia* cysts, whether they be from the dog population or human populations, or whether they are prevalent in certain parts of town or in certain water supplies.

# Axenically Cultivated *Giardia lamblia:* Growth, Attachment and the Role of L-cysteine

**Frances D. Gillin and Louis S. Diamond**
*Laboratory of Parasitic Diseases*
*National Institute of Allergy and Infectious Diseases*
*National Institutes of Health*
*Bethesda, Maryland*

*Giardia lamblia* is grown axenically in vessels nearly filled with medium and with little air phase. The media used are undefined, but all contain added L-cysteine, probably as a reducing agent. We have studied the requirement for L-cysteine and its effects upon the kinetics of trophozoite multiplication, sensitivity to air, and attachment to the culture vessel in Diamond's TP-S-1 and new TYI-S-33 media using a strain of *G. lamblia* isolated by E. A. Meyer and obtained from G. Visvesvara and G. R. Healey.

If L-cysteine was omitted from either medium, trophozoites did not grow, and eventually lysed. If L-cysteine were required only as a reducing agent, it should be replaceable by other reducing agents. In TYI-S-33 medium, the requirement for L-cysteine was rather specific: only glutathione or N-acetyl-L-cysteine supported slight growth. In TP-S-1 medium, lower concentrations of L-cysteine satisfied the requirement and other sulfhydryl compounds were partially effective: possibly because of higher endogenous L-cysteine concentrations in this medium. Ascorbic acid was totally ineffective.

Rates of killing of trophozoites in the absence of L-cysteine, were determined using a new quantitative method. Trophozoites were exposed to various experimental conditions: (1) high or low oxygen tension ($pO_2$) achieved by varying the relative liquid to air volume; and (2) presence or absence of L-cysteine or D-ascorbate. After varying times, survival was measured by clonal growth in a semi-solid agarose medium in culture tubes with low $pO_2$ plus L-cysteine. Survival curves obtained under low $pO_2$ ($\leqslant 30$ mm Hg) were complex; 50% of the cells were killed in ~ 3-1/2 hours with neither L-cysteine nor D-ascorbate and in ~ 11 hours with only D-ascorbate. Under increased $pO_2$ (~ 140 mm Hg), trophozoites were killed exponentially ~ 50%/hour without L-cysteine or D-ascorbate. With *both* L-cysteine and D-ascorbate, killing at the same rate was preceded by a lag of ~ 11 hours, while with either one the lag was ~ 5 hours. Thus, while D-ascorbate did not support growth of trophozoites, it did provide some protection from killing by oxygen.

*G. lamblia* trophozoites in axenic culture exhibit a striking tendency to attach to the walls of the culture vessel in addition to their ability to swim freely. Attachment of trophozoites to intestinal epithelium in vivo may play a role in the disease process. The proportions of attached and free trophozoites were determined during growth of cultures in TP-S-1 initiated (1) with only free cells or (2) with cells which had been attached, but were detached by chilling. In both cases, a majority of the cells was attached until the end of logarithmic growth phase.

Attachment of trophozoites to glass in TP-S-1 with L-cysteine was rapid for the first 2 hours then continued more slowly. With only D-ascorbate, attachment was somewhat depressed even before the cells began to show decreased viability. Cells did not attach without L-cysteine or D-ascorbate.

Thus, L-cysteine appeared to fullfill at least 2 requirements in the axenic cultivation of *G. lamblia:* first as a reducing agent; and second as a growth factor- possibly as an essential amino acid.

## Discussion

L. DIAMOND: These 2 media were designed originally for *E. histolytica,* and there is a pitfall: in relation to *E. histolytica,* there is not much difference whether you sterilize by filtration or autoclaving, but apparently sterilization by autoclaving TP-S-1 or TYI-S-33 will result in no growth of *Giardia.*

G. HEALY: I would like to commend you for a nice job. It was an elegant series of experiments, and I can appreciate the work that has gone into it, realizing the short period of time you had the cultures.

I can see problems in the future; the realization that people will soon be discovering new trophozoites from cysts by the elegant work performed, and that more cultures will soon be established. Having suffered the admonitions of Dr. Diamond in naming *E. histolytica* strains, and seeing the incredible mixup in naming *Acanthamoeba* and *Naegleria* isolates, I would make a plea that Dr. E. Meyer, Dr. L. Diamond and Dr. Visvesvara get together and work out some system for naming these isolates. I do not even know what designation you use for this one except that it is something that Dr. Meyer cultured after it was removed from somebody's duodenum.

F. GILLIN: Dr. P. Daggett of the American Type Culture Collection said that when the culture from Dr. Meyer's lab was delivered he filled in a blank for the designation of the strain and Portland 1 was written down.

G. HEALY: That proves the point that a system is needed for naming these isolates.

E. MEYER: What per cent agar did you use in your semisolid medium?

F. GILLIN: First of all, it is agarose and it is a very low per cent, about 0.2, so it really is semisolid. G. Lakhonina and J. Teras (Estonian Academy of Science; Abs. 5th Intern. Congress of Protozoology, p. 188, 1977) used a really solid 1.5 per cent agar medium.

E. MEYER: Yes, and the results were quite different from ours. They observed that the organisms were confined to the interface between the medium and the glass.

F. GILLIN: I was not aware of that, but that does not surprise me at all. I think that inside such a solid agar matrix they really could not grow very well. I might add that we see localized colonies within the agar matrix and eventually there are halos between the agarose and the glass so that cells are escaping. This does lead to potential problems in obtaining clones, which can probably be overcome by dilution.

S. ERLANDSEN: Do you have any idea how many organisms you have to have in order for the colonies to become visible?

F. GILLIN: You mean how many cells constitute a colony?

S. ERLANDSEN: Yes, and the fact that there may be many colonies that you are not detecting because you cannot see them if you are just using a macroscopic examination.

F. GILLIN: We have what we call colony forming or cloning efficiency in which you take the number of cells that you put in, which is a statistical figure arrived at by dilution from a counted sample, and divide by the number of colonies you count. You get a perfectly linear dose-response curve. The colony forming efficiency is in the range of 30 to 70%, usually about 50%.

S. ERLANDSEN: There's about 30 to 50% that you sometimes do not detect, and the question is whether they reproduce or whether they remain viable and are not multiplying to give a visible colony.

F. GILLIN: Most bacteria or trichomonads have a 100% colony forming efficiency. That is not the case here. I worked out a similar method for growing *E. histolytica* trophozoites in agar. The *histolytica* trophs prefer agar to agarose, and they prefer a slightly higher concentration, about 0.5%. You can look at the agar culture under an inverted microscope and estimate the number of cells in a visible colony of *E. histolytica*, which is of course a much larger cell. You may see single cells but if you let the culture grow longer, the colony counts do not increase with time. I really do not have a very good idea of how many cells there are per *Giardia* colony.

# SESSION VII - RESEARCH NEEDS

*Chairman - Gordon G. Robeck,*
*U.S. EPA, MERL, Cincinnati, Ohio*

## Giardiasis and the Safe Drinking Water Act

**C. Hendricks**

## Panel Discussion: Current Research and Future Research Needs

*Moderator - G. G. Robeck, U.S. EPA*

**PANEL MEMBERS**
**L. J. McCabe, U.S. EPA**
**M. G. Schultz, DHEW, CDC**
**T. M. Vernon, Jr., Colorado Department of Health**
**K. Kawata, Johns Hopkins University**

# Giardiasis and the Safe Drinking Water Act

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## ABSTRACT

Historically, regulatory activity in the United States with respect to drinking water has focused on the containment of infectious disease. The Safe Drinking Water Act of 1974 and its 1977 amendments are no exception. However, they do provide the U.S. Environmental Protection Agency with additional tools to control waterborne disease including options for establishing maximum contaminant levels or mandatory treatment requirements. Based upon analyses of these regulatory means of controlling contaminants, it is possible to regulate infectious contaminants such as *Giardia* on a case by case basis, where risk can be established.

This technical symposium to review the waterborne transmission of giardiasis has afforded the Agency, the Department of Health, Education and Welfare, the waterworks industry and various individuals from universities, state organizations, as well as the public, a unique opportunity to investigate one of the microbiological agents involved in waterborne gastroenteritis. The National Academy of Science volume, "Drinking Water and Health," drew attention to what appeared to be a steady increase in waterborne disease since the early 1950's. Whether this "increase" is due to actual disease, improved reporting practices or both, is debatable; but, the opportunity we have had here can be of major significance in determining a means of control of infectious disease.

This report will center on the Safe Drinking Water Act, its provisions and how it is currently being implemented to control pathogenic microbiological agents. I will also discuss a series of regulatory options, any of which could be taken under the Safe Drinking Water Act. Finally, I will suggest research that is needed to answer fundamental questions concerning the treatment of drinking water for the control of *Giardia lamblia* and other pathogenic microorganisms.

## LEGISLATIVE CONTROL OF DRINKING WATER

The concept of legislative control of infectious disease in the United States began with the signing of the National Quarantine Act of 1878. This Act was designed to prevent the introduction of infectious or contagious diseases into the United States. Modifications of the National Quarantine Act in 1890 and 1893 provided the Treasury Department with authority to prevent the introduction of contagious diseases across state lines and granted the Department additional quarantine powers. The first Interstate Quarantine

Regulations based on this legislation were published in 1894. Under these regulations, activity primarily consisted of maintaining sanitary drinking water aboard vessels. However, in 1912, due to severe outbreaks of intestinal disease among passengers aboard steamships on the Great Lakes, the Treasury Department issued Circular Number 49 which required U.S. Public Health Service surveillance of sanitary conditions on interstate carriers. Paragraph 15 was added to the Interstate Quarantine Regulations at this time which required State certification that drinking water aboard interstate vehicles must be incapable of causing disease. This paragraph, in concept, was the yardstick by which the 1914 Treasury Standards for drinking water were developed. The 1914 Treasury Standards prescribed mandatory limits for bacteria and were applicable to about 9,000 interstate carrier supplies. In addition to a limit of less than 2.2 coliform bacteria/100 cc, general populations of bacteria were not to exceed 100/cc. Later revisions by the Treasury Department and by the U.S. Public Health Service in 1925, 1943-1946 and 1962 added various chemical constituents to the Standards as guidelines. In 1925 the standard plate count requirement was dropped when a turbidity standard was established as an esthetic parameter.

Early in 1968 the first series of bills was introduced in Congress for the protection of all public water supplies. Eventually the Safe Drinking Water Act of 1974 emerged after 14 attempts. Although Public Law 93-523 amends the Public Health Service Act (P.L.- 410), it applies to all public water systems serving 25 persons or more on a regular basis, or an estimated 155,000,000 Americans. A more complete review of the history of drinking water standards development may be found in Taylor, 1977 (1).

## The Safe Drinking Water Act

With the passage of the Safe Drinking Water Act, Congress mandated the U.S. Environmental Protection Agency (EPA) to provide safe drinking water and to protect the public from waterborne illness and disease. With respect to drinking water standards, Congress intended that the Act be implemented in three separate phases. First, the EPA was to promulgate Interim Primary Drinking Water Regulations (2) based on a revision of the 1962 Public Health Service Standards. These regulations were issued December 24, 1975, and became effective June 24, 1977. Second, EPA was directed to contract with the National Academy of Sciences (NAS) for a two-year investigation of all contaminants in drinking water that could be harmful to man. This report was completed and a summary was published in the Federal Register July 11, 1977. Third, EPA was directed to promulgate more comprehensive revised drinking water regulations based upon the NAS study, current available data and the results of the Agency's on-going research program. These regulations are to be proposed for public comment next year and will not become effective until 18 months after promulgation.

Besides these requirements, the Safe Drinking Water Act of 1974 provides for:

1. The development of national primary drinking water regulations for contaminants that may adversely affect the public health which will:
    a. cover public water systems and protect health to the maximum extent feasible;
    b. include a Maximum Contaminant Level (MCL) for each contaminant or a specified treatment technique if it is not feasible to monitor the level of that contaminant;
    c. contain criteria and procedures to assure compliance; and
    d. may be enforced by the Administrator of the U.S. Environmental Protection Agency if the States elect to not enforce them.
2. The Administrator to take emergency action if a contaminant presents an imminent or substantial hazard to the public.
3. The development of non-Federally enforceable secondary regulations as guidance to the States.
4. The establishment of Federal State programs to improve surveillance over drinking water quality and protect underground sources of drinking water.
5. The authorization for Federal grants to assist the State surveillance and enforcement provisions of the Act.
6. The authorization of variances and exemptions to primary regulations.

In keeping with the precedent established in implementing the 1912 revision of the Interstate Quarantine Regulations, Congress envisioned that the States would exercise primary enforcement responsibility (primacy) over water supplies under their jurisdiction, with EPA assuming this task only when a State was unable or unwilling to meet minimum requirements contained in the Act and associated regulations. In order to attain primacy under the Act, the States must: establish drinking water standards and institute procedures for variances and exemptions at least as stringent as the national regulations; adopt and implement an adequate enforcement program; maintain records and submit reports as required by the Administrator of the EPA; and, establish an emergency response plan and a program for plan review.

### National Interim Primary Drinking Water Regulations

The Interim Regulations establishes MCL's for coliform bacteria, turbidity, ten inorganic chemicals, six pesticides, and radiological contaminants (Table 1). As of June 24, 1977, water utilities are required to conduct periodic monitoring at a prescribed frequency to ensure compliance with the regulations and to notify the public if the standards are exceeded. The Act also provides that the States will develop their own regulations that are at least as stringent; some States have more stringent requirements than those in the Interim Primary Regulations.

The Maximum Contaminant Levels for coliform bacteria and turbidity are essentially those contained in the 1962 Public Health Service Standards.

*Table 1. The maximum contaminant levels for constituents in the National Interim Primary Regulations*

| Constituent | Level (mg/l unless specified) |
|---|---|
| **Biological Parameters** | |
| Coliform bacteria | 1 per 100 ml (mean) |
| Turbidity | 1 NTU (Waiver to 5 NTU possible) |
| **Inorganic Chemicals** | |
| Arsenic | 0.05 |
| Barium | 1 |
| Cadmium | 0.010 |
| Chromium | 0.05 |
| Lead | 0.05 |
| Mercury | 0.002 |
| Nitrate (as N) | 10 |
| Selenium | 0.01 |
| Silver | 0.05 |
| Fluoride | 1.4-2.4* |
| **Organic Chemicals** | |
| Endrin | 0.0002 |
| Lindane | 0.004 |
| Methoxychlor | 0.1 |
| Toxaphene | 0.005 |
| 2.4-D | 0.1 |
| 2.4, 5-TP Silvex | 0.01 |
| **Radionuclides** | |
| Radium 226 and 228 (combined) | 5 pCi/l |
| Gross alpha particle activity | 15 pCi/l |
| Gross beta particle activity | 4 millirem/year |

* Based on annual average air temperature.

The maximum contaminant level for coliform bacteria is 1 coliform/100 ml of water, however a supply may substitute chlorine residual determination for 75% of the required coliform analyses upon receiving state permission. Supplies using this provision must maintain a 0.2 mg/l free chlorine residual. The turbidity MCL is 1 Nephelometric turbidity unit (NTU) with the exception that the State may allow up to 5 NTU if the water does not:

1. interfere with disinfection;
2. prevent maintenance of an effective disinfectant agent throughout the distribution system; or
3. interfere with microbiological determinations.

It is generally agreed that these MCL's are sufficient to protect the public from infectious disease transmitted by recent fecal contamination; however, there are data to suggest that the coliform testing procedure may be not entirely effective in certain diseases of viral and protozoan etiologies. The apparent problem with the indicator procedures is not whether these particular disease producing organisms are found in fecal material, but rather that a number of viruses and protozoans can survive in water for longer periods than the coliform indicators. Some viruses and protozoans also seem more resistant to disinfection than coliforms. For these reasons, spurious positive coliform results must be fully evaluated before their validity is doubted.

## THE PHILOSOPHY OF STANDARD SETTING

It is profitable at this point to consider the decision-making process of setting standards for microorganisms. The Safe Drinking Water Act is quite explicit regarding means the Administrator of the EPA should use to regulate contaminants. The regulatory means open to the Administrator consist of either the establishment of an MCL or a mandatory treatment technique or techniques. Although MCL's can be developed for specific types of water systems (ground, surface, large or small), there is considerable flexibility in specifying treatment for supplies where the population is at risk. In both cases however EPA must demonstrate that: 1. there is a need for regulation, and 2. the regulatory action is economically and technologically feasible.

The determination of whether a contaminant should be regulated is often a difficult and arduous task. The basic questions in determining human risk are: 1. What is the toxic or infectious potential of the contaminant, and 2. What is the probability of human exposure? Dr. D. V. Subrahmanyan (3), while with the World Health Organization's Community Water Supply and Sanitation Section, has suggested the following criteria on which to base numerical limits:

1. Severity of adverse effects upon the population; irreversible or chronic effects; those which have genetic, carcinogenic, or teratogenic effects or which may be embryo toxic.
2. Persistence in the environment and resistance to environmental degradation; accumulation in man or in the food chain.
3. Metabolic degradation or synthesis in biological systems which may produce metabolites either more or less toxic than the parent compound.
4. Ubiquity and abundance of the agent in man's environment particularly those occurring naturally or produced by man.
5. Size, type and demographic characteristics of the population exposed and magnitude of exposure.
6. Selective exposure of highly vulnerable groups of the population.

### Regulatory Decision Making

A typical decision-making pathway for establishing primary drinking water standards is given in Fig. 1-3. This pathway rather quickly provides administrative guidance on when a constituent should be considered for regulation, the scientific evidence required, the technical and economic considerations to be made, and the social acceptability of the proposed standard. Although a similar decision pathway was used effectively by the Office of Drinking Water in developing the Interim Primary Regulations, these types of decision pathways lack flexibility in dealing with contaminants that may pose substantial risks only at select water supplies. It should be noted that the pathway presented here is highly stylized and individual activities may occur simultaneously with other actions noted earlier in the flow chart.

### Preliminary Action

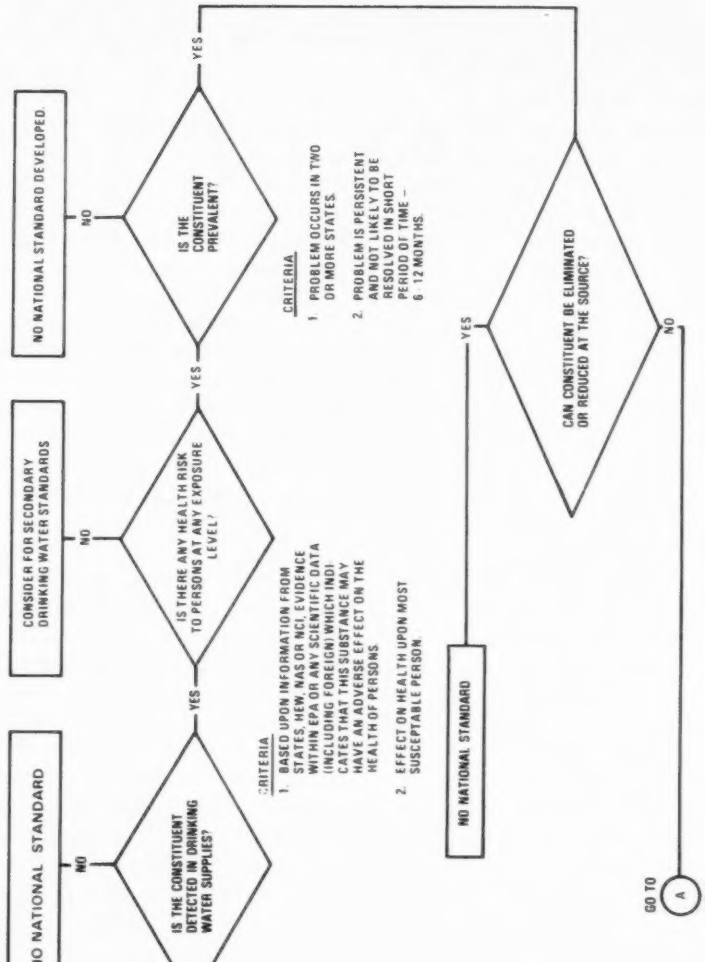
Regulatory action under the Safe Drinking Water Act is predicated upon finding the contaminant in drinking water. Figure 1 describes the preliminary action needed to determine whether a constituent should be extensively considered for possible regulation. Information is gathered from all available sources concerning the public health risk of a constituent and its prevalence. Perhaps the major decision point at this stage in the evaluation is to determine if the constituent can be controlled at the point of origin before source water becomes contaminated. For example, when chlorine is used in disinfection of plant sewage effluents it is effective in reducing or eliminating those constituents. Obvious difficulties arise from non-point sources such as wastes from home sewage treatment facilities or wastes from animals and humans along a watershed. Therefore, consideration must be given to other viable avenues open to the Agency for the control of microorganisms such as the National Pollutant Discharge Elimination System (NPDES) permit program.

### Technical Evaluation

Decisions that must be made in setting standards regarding health risk of a contaminant to the public, and the associated costs with both monitoring and removal of a contaminant, is potentially the most controversial area. Besides the capital, operation and maintenance costs, one must also consider those costs to the public as a direct result of illness. It is important to note that health standards in the Interim Primary Regulations are based upon levels at which no adverse health effects are observed, with an appropriate safety factor included. Although the Administrator has authority to establish an MCL for a contaminant, it is possible that some deleterious contaminants may not be regulated because the risk to the public is not perceived to be great enough to warrant the costs. In addition to these concepts, Figure 2 also shows the decision protocol for determining monitoring and treatment capability as a means of controlling a contaminant.

### Administrative Action

If overall costs have been found acceptable and a tentative MCL can be determined, a monitoring survey may be ordered to determine an estimate of national risk and to gain additional cost information. Information about analytical methods and treatment efficiency is also obtained in the survey. Should the monitoring survey indicate that data for another regulated constituent indicates that the two constituents respond similarly, consideration will be given to a surrogate MCL (Fig. 3). The coliform test in


NO NATIONAL STANDARD
NO
IS THE CONSTITUENT DETECTED IN DRINKING WATER SUPPLIES?
YES
CONSIDER FOR SECONDARY DRINKING WATER STANDARDS
NO
IS THERE ANY HEALTH RISK TO PERSONS AT ANY EXPOSURE LEVEL?
YES
NO NATIONAL STANDARD DEVELOPED.
NO
IS THE CONSTITUENT PREVALENT?
YES
NO NATIONAL STANDARD
YES
CAN CONSTITUENT BE ELIMINATED OR REDUCED AT THE SOURCE?
NO
GO TO
A
FIG 2.

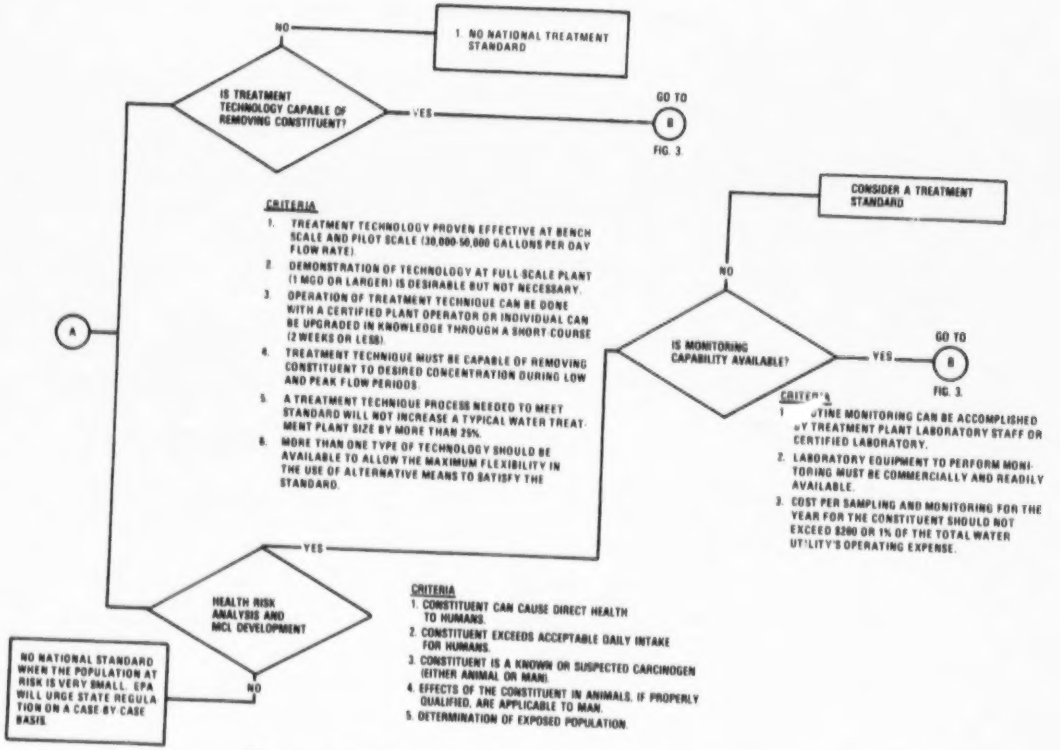
CRITERIA

1. BASED UPON INFORMATION FROM STATES, HEW, NAS OR NCI, EVIDENCE WITHIN EPA OR ANY SCIENTIFIC DATA (INCLUDING FOREIGN) WHICH INDICATES THAT THIS SUBSTANCE MAY HAVE AN ADVERSE EFFECT ON THE HEALTH OF PERSONS.
2. EFFECT ON HEALTH UPON MOST SUSCEPTABLE PERSON.

CRITERIA

1. PROBLEM OCCURS IN TWO OR MORE STATES.
2. PROBLEM IS PERSISTENT AND NOT LIKELY TO BE RESOLVED IN SHORT PERIOD OF TIME – 6-12 MONTHS.

FIG. 1. *Decision-making pathway for primary drinking water regulations—preliminary action.*


IS TREATMENT TECHNOLOGY CAPABLE OF REMOVING CONSTITUENT?
NO
1. NO NATIONAL TREATMENT STANDARD
YES
GO TO
B
FIG. 3.
A
CONSIDER A TREATMENT STANDARD
NO
IS MONITORING CAPABILITY AVAILABLE?
YES
GO TO
B
FIG. 3.
YES
HEALTH RISK ANALYSIS AND MCL DEVELOPMENT
NO
NO NATIONAL STANDARD WHEN THE POPULATION AT RISK IS VERY SMALL. EPA WILL URGE STATE REGULATION ON A CASE-BY-CASE BASIS.

CRITERIA

1. TREATMENT TECHNOLOGY PROVEN EFFECTIVE AT BENCH SCALE AND PILOT SCALE (30,000-50,000 GALLONS PER DAY FLOW RATE).
2. DEMONSTRATION OF TECHNOLOGY AT FULL-SCALE PLANT (1 MGD OR LARGER) IS DESIRABLE BUT NOT NECESSARY.
3. OPERATION OF TREATMENT TECHNIQUE CAN BE DONE WITH A CERTIFIED PLANT OPERATOR OR INDIVIDUAL CAN BE UPGRADED IN KNOWLEDGE THROUGH A SHORT COURSE (2 WEEKS OR LESS).
4. TREATMENT TECHNIQUE MUST BE CAPABLE OF REMOVING CONSTITUENT TO DESIRED CONCENTRATION DURING LOW AND PEAK FLOW PERIODS.
5. A TREATMENT TECHNIQUE PROCESS NEEDED TO MEET STANDARD WILL NOT INCREASE A TYPICAL WATER TREATMENT PLANT SIZE BY MORE THAN 25%.
6. MORE THAN ONE TYPE OF TECHNOLOGY SHOULD BE AVAILABLE TO ALLOW THE MAXIMUM FLEXIBILITY IN THE USE OF ALTERNATIVE MEANS TO SATISFY THE STANDARD.

CRITERIA

1. ROUTINE MONITORING CAN BE ACCOMPLISHED BY TREATMENT PLANT LABORATORY STAFF OR CERTIFIED LABORATORY.
2. LABORATORY EQUIPMENT TO PERFORM MONITORING MUST BE COMMERCIALLY AND READILY AVAILABLE.
3. COST PER SAMPLING AND MONITORING FOR THE YEAR FOR THE CONSTITUENT SHOULD NOT EXCEED $200 OR 1% OF THE TOTAL WATER UTILITY'S OPERATING EXPENSE.

CRITERIA

1. CONSTITUENT CAN CAUSE DIRECT HEALTH TO HUMANS.
2. CONSTITUENT EXCEEDS ACCEPTABLE DAILY INTAKE FOR HUMANS.
3. CONSTITUENT IS A KNOWN OR SUSPECTED CARCINOGEN (EITHER ANIMAL OR MAN).
4. EFFECTS OF THE CONSTITUENT IN ANIMALS, IF PROPERLY QUALIFIED, ARE APPLICABLE TO MAN.
5. DETERMINATION OF EXPOSED POPULATION.

FIG. 2. *Decision-making pathway for primary drinking water regulations—technical evaluation.*

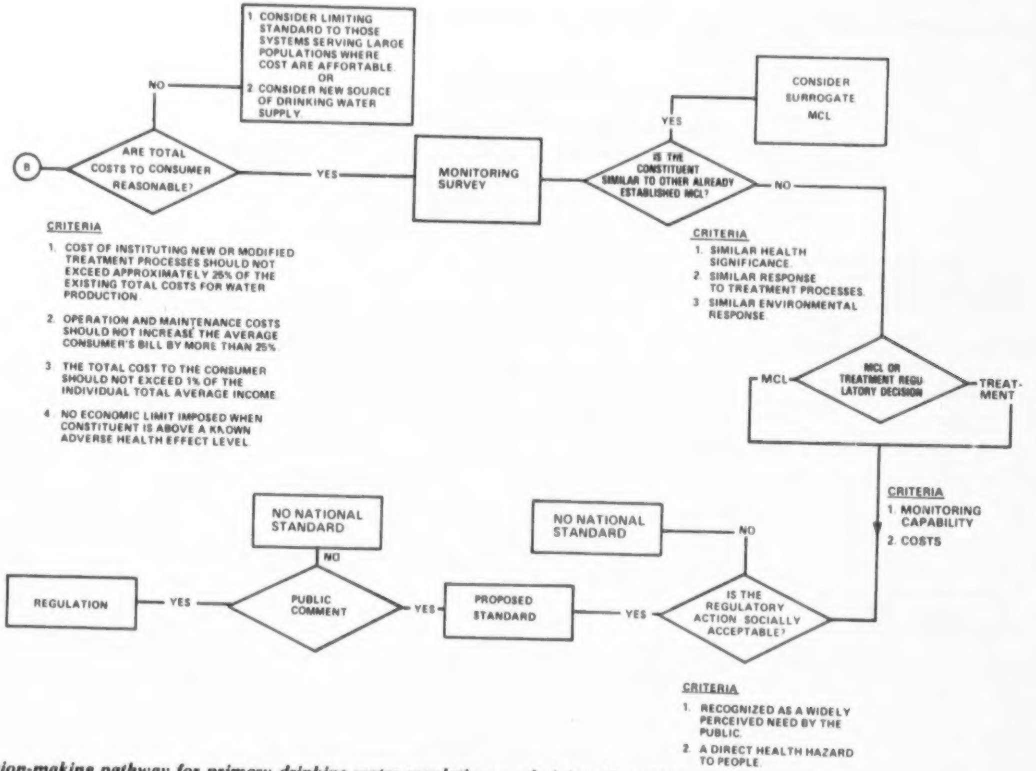

B
ARE TOTAL COSTS TO CONSUMER REASONABLE?
NO
1. CONSIDER LIMITING STANDARD TO THOSE SYSTEMS SERVING LARGE POPULATIONS WHERE COST ARE AFFORTABLE.
OR
2. CONSIDER NEW SOURCE OF DRINKING WATER SUPPLY.
YES
MONITORING SURVEY
CRITERIA
1. COST OF INSTITUTING NEW OR MODIFIED TREATMENT PROCESSES SHOULD NOT EXCEED APPROXIMATELY 25% OF THE EXISTING TOTAL COSTS FOR WATER PRODUCTION.
2. OPERATION AND MAINTENANCE COSTS SHOULD NOT INCREASE THE AVERAGE CONSUMER'S BILL BY MORE THAN 25%.
3. THE TOTAL COST TO THE CONSUMER SHOULD NOT EXCEED 1% OF THE INDIVIDUAL TOTAL AVERAGE INCOME.
4. NO ECONOMIC LIMIT IMPOSED WHEN CONSTITUENT IS ABOVE A KNOWN ADVERSE HEALTH EFFECT LEVEL.
IS THE CONSTITUENT SIMILAR TO OTHER ALREADY ESTABLISHED MCL?
YES
CONSIDER SURROGATE MCL
NO
CRITERIA
1. SIMILAR HEALTH SIGNIFICANCE.
2. SIMILAR RESPONSE TO TREATMENT PROCESSES.
3. SIMILAR ENVIRONMENTAL RESPONSE.
MCL OR TREATMENT REGULATORY DECISION
MCL
TREATMENT
CRITERIA
1. MONITORING CAPABILITY
2. COSTS
IS THE REGULATORY ACTION SOCIALLY ACCEPTABLE?
NO
NO NATIONAL STANDARD
YES
PROPOSED STANDARD
YES
PUBLIC COMMENT
NO
NO NATIONAL STANDARD
YES
REGULATION
CRITERIA
1. RECOGNIZED AS A WIDELY PERCEIVED NEED BY THE PUBLIC.
2. A DIRECT HEALTH HAZARD TO PEOPLE.

FIG. 3. *Decision-making pathway for primary drinking water regulations—administrative action.*

determining the microbial quality of water is probably the best known example of the use of a surrogate analytical procedure to warn of possible risk of intestinal disease outbreaks.

Some indication of how the public perceives the need for a proposed regulatory action must be obtained since social acceptability is an important determinant in governing our behavior. Should the public view a particular regulation as not acceptable, perhaps we have not followed through with our responsibility to keep the public informed of the risks associated with a particular contaminant. There are, however, instances where the public is fully aware of a problem and is still willing to accept risks. Examples of this include the use of tobacco and certain artificial sweeteners, the disregard by some of the beneficial aspects of fluoride, and even disregard for the 55 mph speed limit.

## REGULATORY BASIS FOR THE CONTROL OF *GIARDIA* AND OTHER INFECTIOUS MICROORGANISMS

Based upon the literature (5-7) the numerous reports of *Giardia* outbreaks (4,8-11), and the information presented at this Symposium, some type of regulatory action may be needed to eliminate or greatly reduce the risk of waterborne giardiasis. There are several regulatory options that are available for the control of organisms such as *Giardia* and these options include the following:

1. Rely on the present coliform standard and/or encourage more use of the chlorine residual substitution procedure.
2. Establish either an MCL or a surrogate for *Giardia*.
3. Establish a regulatory treatment technique for removal or inactivation of *Giardia* in vulnerable supplies.
4. Require vulnerable supplies to be monitored.
5. Warn physicians to be alert.
6. Do nothing at this time and await developments.
7. Request the Administrator to take emergency action as in the case of asbestos at Duluth, Minnesota.

While there is merit in some of the options, none are entirely satisfactory by themselves. An MCL established for all public surface water supplies is possible but there are serious economic drawbacks to it. Monitoring procedures are available for *Giardia*, however routine monitoring would be extremely expensive and require highly trained laboratory technical personnel that appear to be out of reach for most utilities at this time (12,14). Neither surrogate tests for *Giardia* nor increased reliance on coliform bacteria indicator systems seems to be of any real value at this time.

There is merit in the option of requiring the installation of conventional filtration for the control of *Giardia*. In addition to the obvious benefits derived from the removal of cysts and ova of protozoans, the reduction in hazards associated with virus, turbidity and certain chemicals could be expected with that additional public health barrier(13). Small surface supplies, however, appear to be at greatest risk from *Giardia* and the additional construction, operation and maintenance costs associated with a mandatory treatment requirement applicable to all supplies could be burdensome even though necessary. Options 4 and 5 seem sensible steps for States to take under their own drinking water program. Action under the Administrator's prerogatives could be effective should the situation become more than presently perceived.

### Infectious Disease

As stated previously, I believe some sort of regulatory action at the Federal level for the control of giardiasis is appropriate. I do not believe a generally applicable MCL for *Giardia* per se would be the most appropriate approach since the monitoring frequency undoubtedly would be quite low because of economic considerations. Instead, I prefer regulatory action that would specify mandatory treatment if a supply, and, therefore the public, was shown to be at risk from infectious disease. In this way, action could be taken if the supply was vulnerable to a disease producing agent for which treatment could be specified.

Clearly, in a proposal such as this, consideration must be given as to how one determines whether a supply is at risk and an evaluation of the likelihood that people may become ill. The relationship between microorganisms and disease is fundamental, but I can suggest to you some determining factors that could lead to the designation of supplies requiring treatment or a modification of their present treatment scheme. These include the following:

1. Has a supply been incriminated in an outbreak of waterborne disease?

   Important in this consideration would be whether the problem resulted from source water, the treatment facility or the distribution system since the nature of the problem would trigger specific corrective treatment.
2. Have pathogenic organisms been recovered from the source water, demonstrated on the watershed, found in the raw water intake, or in finished water?
3. Has a State or Federal sanitary survey determined that a supply is at risk? Is the supply properly operated?
4. Is the supply in continued violation of State and Federal drinking water regulations for coliform bacteria and turbidity?
5. Is the supply in compliance with existing State and/or Federal guidelines?

I am sure there are other items that could be added to the list and those presented here could be sharpened considerably, but based upon these considerations, research is needed in several areas. Specifically, these recommendations were designed for *Giardia*, but they are more generally applicable to research required for viruses and other protozoa:

1. Develop and define treatment techniques for the removal or inactivation of *Giardia* cysts.
2. Develop a rapid, inexpensive monitoring procedure for *Giardia* cysts.

3. Investigate the feasibility of an indicator system.
4. Perform studies to determine:
   a. effect of the environment on the survival of cysts and trophozoites;
   b. mechanisms of disease transmission;
   c. vulnerable human populations; and
   d. how to establish populations at risk.

In conclusion, I think there are alternatives to developing MCL's and mandatory treatment requirements applicable to all or even particular classes of water. The development of rules specific for supplies shown to be at risk would have an added advantage of being flexible enough to adapt to local conditions while keeping local and state costs to a minimum. I would encourage all of you to study this proposal and I look forward to your comments as we move forward together in the implementation of the Safe Drinking Water Act.

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## Discussion

L. McCABE: You said one of the control options was to request the Administrator to take emergency action. Could that be a selective option exercised by him each time there is an outbreak?

C. HENDRICKS: That is one of the options listed in the Safe Drinking Water Act. As I would interpret it, in the case of an emergency where action is needed rapidly without going through the complicated regulatory procedure, the Administrator upon receiving a proper basis for a decision could then move to take action that was recommended to him or action that he may decide upon himself. I do not view it as a means of acting on every outbreak that crops up. If, for example, suddenly there were a tremendous epidemic involving deaths or very serious illness, then I suspect the Administrator would move very rapidly. But I do not view it as a practical means for controlling giardiasis unless the situation should deteriorate substantially.

D. PRICE: The people of Florida are concerned because we have been told, or it has been suggested, that by the year 2000 the southeastern United States may be the only resource for fresh water for many parts of the United States. Since the reduction of the chlorine content in water to 0.2 milligrams is a reasonable amount, we are finding that throughout Florida now the coliform counts are well over the basic limits, and no one seems to know exactly what to do about this. I realize there is also a problem with trihalomethanes with the higher levels of chlorine. I would appreciate any comments you might make on this. It also seems to me that any safe drinking water regulation must be tied to some extent to the volume of water used in the particular community and to waste disposal methods and effluent evaluations and to the type of water purification system that could be employed under the circumstances. I also would like to know at what level the decision really can be made that there is a hazardous situation.

C. HENDRICKS: First of all, the Safe Drinking Water Act is applicable at the tap. We are concerned with source water and concerned with treatment and distribution. However, the MCL's are applicable at the tap, and we must rely upon other regulations to protect source water. According to the Act, source water standards are to be considered when treatment techniques are prescribed.

With respect to trihalomethanes, before September 1, I would have been glad to talk to you at length about them. However, I am legally bound not to discuss those proposed regulations now that the comment period has closed.

G. ROBECK: I think it is fair to say there is no regulation that says chlorine has to be limited to a residual of 0.2. That is a matter of option on the part of the state agency with primacy. I do not think you could say that the sudden increase is due to that regulation. There may be some people who want to taper off in the amount of chlorine. In Florida they used to advocate 0.5 parts free available chlorine and now they have reduced that. That may be partly the cause, but I do not think the regulation per se is the cause.

# Panel Discussion
# Current Research and Future Needs

*Moderator: Gordon G. Robeck, USEPA*

**PANEL MEMBERS**

**Leland J. McCabe, USEPA**
**Myron G. Schultz, DHEW, CDC**
**Tom M. Vernon, Jr.,**
**Colorado Dept. Of Health**
**Kaz Kawata, Johns Hopkins University**

G. ROBECK: In an effort to bring some focus to this Symposium, we thought it would be advisable to have four representatives with different perspectives share with us their ideas on what the research needs might be in general, and particularly of course for the agency. We have, therefore, asked federal representatives from EPA and from CDC, a state representative and a university researcher to speak to us on how they see the research needs in their areas. There will be opportunity for discussion from the floor.

Our first panelist is Leland McCabe who is the Director of the Field Studies Division in the Health Effects Research Laboratory. He has been responsible for directing a good share of the health effects research in the USEPA on drinking water for many years and has been involved for a decade in the general area of epidemiology as well as sanitary engineering. He is well qualified to speak from the perspective of USEPA.

L. McCABE: It would seem that we have about four areas in which EPA has research needs. I think there is clearly a public health problem that we cannot tolerate. Certainly the outbreaks in Portland or Rome were of sufficient magnitude to indicate that those outbreaks have to be stopped. So I would put as our first research need to prevent the occurrence of waterborne *Giardia* outbreaks, and some of this research is as mundane as the data that were presented by Ed Lippy and then followed by Al Bingham, like how long you have to boil the water to make sure you can stop an outbreak. We had a lot of discussion about that, but we need definitive data yet on that point. You apparently do not have to boil it for 5, 10 or 15 minutes. You may just have to get it to the boiling point to do the job.

If we are going to prevent these outbreaks from occurring, clearly there will be larger utilities or state regulatory agencies that will want to move ahead before we go through the decision matrix that Dr. Hendricks outlined for us. We should be able, from the research at EPA, to tell them what they have to do to improve treatment. That generates another research need: how to measure the efficacy of treatment.

We have been doing some work in-house on how to get these things out of the water and extramural work at Colorado State University on infectivity. The new excystation work at the University of Oregon certainly holds promise for determining the adequacy of disinfection. If this does not mature very quickly, we may have to drop back to the mouse model or something like that to measure the efficacy of treatment.

In the area of treatment, research has to be linked to EPA's current problem that relates to the production of trihalomethanes from chlorination. We are going to be promoting the use of shorter contact times and lower residuals to prevent the formation of trihalomethanes. These of course may be contraindicated from research on how to get rid of *Giardia*. It is also possible that some of the alternate disinfectants might be much better than we ever thought they would be.

There is a great difference in the type of research that is necessary for the large utilities or for the individual home, and this will have to be considered. The University of Oregon is going to pursue some of the issues on alternate disinfection, so we will get data on how that would fit in since the EPA may change its disinfection recommendations. Dr. Logsdon's data showed we are moving along on how to do filtration. This will also be pursued by the University of Washington through EPA funding.

The next area of research would be outbreak investigations. Water is a very efficient transmitter of disease, even though the main endemic route may be person to person. Contaminated water can obviously infect thousands of people in a short period of time. *Giardia* presents a situation where the source is usually contaminated, so if there is a deficiency it is not like a cross-connection that may affect one block or something like that; it is going to get the whole community if treatment is inadequate.

Certainly we have to do better workups on the outbreaks that do occur. This of course is a primary responsibility of CDC, but EPA is always willing to assist in defining the water situation more specifically. The state regulatory agencies are going to have to take a greater interest in how to do this. In some states EPA, because of the primacy rule, will be the regulatory agency. We ran into an outbreak or two recently where there was a dichotomy in the state concerning who worries about the water. The health department is not that agency any more, so we are going to have to work out some administrative mechanisms to be sure everybody is trying to do the same thing.

We do plan within the next year to have some projects collaborating with CDC on improving reporting of disease outbreaks. We will probably try this out in a few model states with somebody on site in the state that will try to stimulate the reporting of outbreaks so we can get a quicker response. In 50% of the outbreaks the etiology is unknown. We have to work on that situation to understand the epidemiology of the causative agents. We want to be alert to the fact that some disinfection practices will be changing because of other regulations, and we will want to have a quick indication if that is causing more outbreaks and stop them right away if this does occur.

Now, another decision to be made is if we should make everybody do something about the problem under the Safe Drinking Water Act. There are several areas of research, including defining the endemic situation, determining the proportion of persons who are infected but are not sick, and there are also many water supplies that are probably contaminated and do not cause disease.

When I was with CDC, on one occasion we determined the effect of the environment by treating everybody in the community and then measuring their reinfection rate. That is a possibility here. You could clean everybody up and then measure different qualities of water and how fast it takes for reinfection to occur. Certainly the point made on the sewage monitoring is one way we might get a feel for the endemic situation, and it also has a relationship to the next item I will bring up.

As Dr. Hendricks has outlined, if there is a problem, we have to define the scope of the problem and determine the most economical method of treatment, then we must develop cost-benefit information. In doing monitoring, as the regulation suggests, we have to have a suitable technique. It does not have to be for the agent itself; we could establish something like we do for pasteurization, a time-temperature relationship to see if the treatment is working all right.

The fourth area involves EPA's effort to change the methods of waste treatment in this country. We are very enamored at the moment with land application, something that we deliberately did not do in the past. With the cyst levels that were indicated yesterday in sewage, there should probably be a big concern for spreading this on the land. It is going to have an ecological impact, and we may start getting quite a few of the wildlife infected. We may then have a geometric sort of progression between animal and man. The nature of the research is split into two areas: how to get rid of the liquid waste and how to get rid of the sludge. Both of them have a spray component, so we get involved with an aerosol exposure. Nobody wants to get a *Giardia* cyst down their throat because of aerosol from the spray irrigation of waste water.

Of course, even if EPA's interests are totally taken care of in the research and the regulatory sense with a good program, there is still going to be a *Giardia* problem because the portion that is waterborne is only some segment of the total. So I think that research in other areas by other agencies may be needed with regard to this organism.

G. ROBECK: Our next panelist is Dr. Myron Schultz. You may recall that he is the Director of the Parasitic Disease Division, Bureau of Epidemiology, Center for Disease Control, DHEW in Atlanta. Dr. Schultz will speak to us from that organizational perspective as well as his professional perspective.

M. SCHULTZ: I would like to begin my comments by applauding EPA for holding this conference. I think it is very timely and beneficial. I would also like to applaud EPA for the degree of cooperation and collaboration with CDC. In my own observation it has been a very salutary collabortion, a very smoothly functioning, good complementing of skills, and it is not always typical of interagency collaboration that I have seen at other times and places.

I think one of the key concepts that I would like to leave with you is the need for surveillance, and we are acting now to increase our surveillance for waterborne diseases both unilaterally and collectively. Some philosopher said that the proper study of mankind is man himself. Well, the proper study of waterborne giardiasis is more and more the study of giardiasis until we satisfy all the academic questions that have been raised. Mr. Craun indicated that there have been 23 recognized outbreaks of giardiasis since 1966, and that is the most common cause of recognized waterborne disease outbreaks. I think that this is just the tip of the iceberg, and there is probably much more going on that we have not yet recognized. We should appreciate that giardiasis does not cause death and does not commonly cause fever, so these two indicators for an epidemic are missing, and the principal symptom, diarrhea, is one that is not commonly reported even though it may be a common symptom. We need to refine our surveillance techniques to get a better fix on this, and I am sure once we do that we will find much more giardiasis.

We have just established a unit within the Epidemiology Bureau of CDC to investigate all waterborne infectious disease, including giardiasis, and it is headed by Dr. Jim Hughes. He and his colleagues were just here at EPA last month to discuss protocols, and I think the sort of collaboration that has existed in the past will be expanded. I think this is needed because there are a lot of questions in epidemics that could be answered, and the place to answer questions about *Giardia* is the epidemic rather than the endemic situation. I think if the disease were only endemic we would not be here today. In the epidemic we want to prevent further occurrence of cases and we want to correct any problems in existing facilities that may be causing it. We want to establish the traditional epidemiologic parameters of time, place and person, and then we want to carry on special studies that would contribute to our understanding of giardiasis.

The specifics of these remain to be elaborated and fixed in common protocols between CDC and EPA, but they all deal with pathogenicity. The other key concept I would like to mention this morning is pathogenesis—the first concept being surveillance—because in all the talks during the past two and a half days on various aspects of giardiasis the issue of pathogenesis came up, and there were more questions raised than answered, as so often occurs in new problems.

Just to mention a few, what is the significance of *Giardia* cysts in a water supply? Does it mean that there is going to be an epidemic or that there was an epidemic? In Rome, New York we had 5000 people infected. We found one *Giardia* cyst in 250,000 gallons of water. Of course, this was somewhat after the occurrence of the epidemic, and this emphasizes the point that we should get in as promptly as we can. Another question in pathogenesis is whether there are any coexistent factors that stimulate *Giardia* to become pathogenic such as viral and other infections.

The role of domestic and wild animals in propagation of the disease needs to be determined. There is a notion of beaver being a reservoir for man, but it remains to be shown what species and strains go through beaver. Of course we are prevented from infecting human volunteers in this day and age with *Giardia*, which is in a way a hindrance to understanding pathogenesis. But there are many questions on reservoirs of *Giardia* for man that could be answered. What is the role of the domestic dog? Will it turn out to be another story like toxoplasmosis? In that case the cat was found to have an important role in the life cycle. Perhaps there is a stage that we are missing here in *Giardia* in domestic animals.

Another question that intrigues me is, if this is a prevalent infection, as we believe it is—in our surveillance work we found that 3.4% of stools examined in state health department laboratories were positive for *Giardia*—why is it that evidence of person-to-person transmission is lacking? I made a point yesterday that in waterborne epidemics that we have seen—Lenigrad, Rome, and other cities—there has been very little secondary transmission on a person-to-person basis, and there is no evidence for foodborne transmission, and we know that in children in nurseries there is person-to-person transmission at quite a rapid rate, but in adults we have seen very sparse evidence for this. I think only through further epidemiologic work can we begin to answer these questions.

Then of course we have the whole human host-parasite relationship. A lot of questions were raised during the conference on how it is manifested. Is it increased virulence of some of the strains? Is it somehow a predisposition? We know that there are a few factors like specific immunodeficiencies, but there are probably many more that account for some people being infected and other people not being infected. Also, what are the factors that account for self-cure? It is not magic; it is some biologic phenomenon at work, and we should research this. Most important of all, why do some people become diseased and other people just become carriers? There are many other questions to be asked but I think I will stop at this point.

G. ROBECK: I would like to go on and ask Dr. Tom Vernon, the Deputy Director of the Colorado Department of Health, to discuss some of these same issues from the State Health Department point of view. Dr. Vernon was educated at Duke, got his medical degree at Harvard Medical School, and has spent at least 3 years with CDC as an epidemiologist, so he has had a variety of experience and training.

T. VERNON: With Colorado playing a leading actor role in the last several days, it has not always been comfortable to be here. A number of people have wondered why Colorado has taken a key role, and I think it is useful, at least epidemiologically, to understand some background to the Colorado situation.

As is very clear from the days of Dr. William Foege, now Director of CDC and once an epidemiologist in the Colorado Department of Health, in his studies of hepatitis, the attack rates were greater in the high altitude mountain counties than they were in the plains counties, my supposition being that water treatment was less adequate. Our smaller towns in the past have depended more on basic purity of the water and less on treatment systems.

Many of us have been misled into thinking that the Coors beer slogan "pure Rocky Mountain spring water" actually means pure Rocky Mountain stream water. Backpackers tend to subscribe to this and drink the surface water, and I think such people in other parts of the country as in the Cascades and the Appalachians, do not drink stream water nearly as much. So the exposure is clearly there in Colorado.

There has been a sensitization in Colorado toward *Giardia* since the Aspen outbreak in 1966. We had an outbreak in '68, one in Golden in '69, Wild Basin Lodge in '71, Winter Park in '71, Boulder was mentioned in '72, the Grand Lake Lodge in '72 and '73, Lake City in '74, and Vail in '78. Giardiasis has been of primary interest to the Colorado Department of Health and our local health departments, and there is a great deal of sensitization not only among the public but particularly among the physicians in the state, so that endemic disease is also very widely recognized.

The issue of the volume of people exposed is another point in the Colorado history. Our population is now 2.6 million. It is larger by a wide margin than any other Rocky Mountain state and is growing by 2.5% a year. If you add to that large population, many of whom enjoy those backpacking trips, the million or more tourists that we have, you can see why giardiasis is a problem.

What are the research needs as we see them? We have told you that in assuming primacy with the Safe Drinking Water Act, we have in our regulations now a requirement for complete treatment of surface waters. That is the phraseology used, and of course this will include for us more than filtration. To meet this requirement additions must be made for 19 municipal water supplies, but that is not the main problem. We are concerned about many smaller, non-municipal water supplies.

We have to think first about the source. What characterizes one surface water source from another? What are the animal species present? We want to continue the work that Hibler and Davies began. What detector systems could we develop, or indicator systems, as Dr. Hendricks used the phrase this morning, to tell us whether or not a given surface water is a problem? What is the relationship of surface water to some so-called ground water sources which are actually only surface water passed through poorly filtering soils? What is the human habitation or recreational use of a given watershed?

What are the seasonal changes in *Giardia* from the water source? What is the relationship to flow? As you might guess, we have a tremendously variable flow seasonally and also from year to year. The difference between 1977 and 1978 in total snow pack in the Rocky Mountains was tremendous such that our flows through late 1977 were way down. We need to look at the treatment systems. Everyone has addressed this as to effectiveness for our municipalities, but we also need to check our non-municipal public water supplies, the motels and lodges which take water out of the Rio Grande and the Colorado Rivers and so forth. What are our choices of filtration media? I was very well educated yesterday on diatomaceous earth, and I think we need to know more, a great deal more, about what works, what combination of filtering media could be put together and what is the best means of coagulation. What is the base line level of giardiasis we can expect in our population which cannot be tracked to direct contamination of raw water? And then of immediate importance and seemingly quite easy to determine, what can we recommend as a disinfectant to our backpackers?

I experienced the end of my Flagyl course this morning, left over from a backpacking trip six weeks ago. I realized either my 2% tincture of iodine did not work at all or I did not let it contact long enough, or I drank from what appeared to be a particularly clear stream. At any rate, we are recommending methods for disinfection to the backpacker in which we have relatively little confidence.

G. ROBECK: We appreciate the personal as well as the official experience and point of view. It brings the Symposium as I said initially, a little more into focus.

To help us dwell a little more on the technology, we have with us Dr. Kaz Kawata who comes to us from Johns Hopkins University where he is the Acting Director of the Division of Environmental Health Engineering. He received his education in various institutions in the U.S. and experience in various places through the world. He was trained in Oregon as a civil engineer, moved on to Minneapolis and Berkeley for more training and received his Ph.D at Johns Hopkins. He comes to us with a great deal of experience in environmental health in the tropics dealing with exotic diseases, which to a certain extent some people think giardiasis to be.

K. KAWATA: I would like to take this assignment from the point of view of the environmental health engineering consultant, because we work in the area of environmental health engineering and there are some questions that I would like to have answered. One of the main answers that I would like to have is how big is the problem if there really is a problem, or if there is a potential problem, what do we have to do as sanitary engineers and environmental health engineers in terms of the treatment of water for the consuming public, or what do we have to do, also, if there are that many cysts being excreted? Chandler & Read (Introduction to Parasitology, John Wiley & Sons, New York, 1961) indicate that one stool from a moderate infection may produce as many as 300 million cysts. Now, if that many cysts from infected people come down to a sewage treatment plant, then is there a problem? What kind of treament is required not only for the liquid effluent that is going out, but also for the sludge?

The American Public Health Association's Handbook on Control of Communicable Diseases in Man (A.S. Benenson, 1975) gives a figure on the prevalence of infection in the neighborhood of 1.5 to 20%. It is said that prevalence is dependent upon the community and the population involved.

If the infection rate is that high, then why isn't there more disease in the community? The answer that was given during the symposium is that perhaps this is an organism that infects many but causes disease in few. If that is the case, what are the host factors that are involved; what is an infective dose? In terms of treatment processes that we have to design, we have to take into consideration the infective dose. Are the factors within the etiologic agent itself? During this Symposium, the biological difference in organisms from different donors was mentioned. We would like to know what those important differences are. It is hard to believe that giardiasis is not prevalent in other areas of the United States besides the mountain areas. What about migrant labor camps?

It is also hard to believe that giardiasis is not foodborne. Perhaps a study into foodborne transmission of giardiasis might be done in a country like India. When I was assigned to India, we had to take our own toilet paper there. It was very difficult in the early 1950's to buy toilet paper in India. Many people in India do not use toilet paper; they wash their perianal regions with their left hand. They take a little bit of water with them when they go to defecate out in the field. We hired some of those people to be our cooks, and we had giardiasis when we were in India. Chandler and Reed stated that there are quite a number of invertebrates that harbor this intestinal flagellate. They said that you can find them in vertebrates ranging from fish to man. Evidently there is much more to be done, much more to be learned in terms of what animals may harbor the organisms that may infect man.

Now, as to control, there is really very little information in the literature for sanitary engineers or designers on treatment processes. We need to know a lot more about the removal of the organism by coagulation, flocculation and sedimentation, and of course also by filtration. In a rapid sand filter plant the bulk of the work is done ahead of the filter, so we need to look at those processes. As for filtration of unconditioned or preconditioned water, the designer needs to have data on the efficiency of cyst removal for varying parameters of sand size and uniformity coefficient, temperature and filtration rate. In doing this, we run into the problem of recovery and enumeration that W. Jakubowski talked about in this conference. We certainly should have a better method for enumeration and recovery.

At Johns Hopkins we have been involved in disinfection studies for a number of years. It would be nice to have a good reliable in vitro cultivation technique for *Giardia* so that we could harvest cysts from culture and then subject them to all kinds of insults with disinfectants. Then, after hurling these insults on the organism, we need to know how to tell a live cyst from a dead cyst. Should we use excystation as proof of survival? If we are going to use excystation, then we want to know the relationship between excystation and viability. We also want to know the relationship between viability and infectivity. We can also go to harvesting cysts from human donors, but getting a good human donor is difficult. We at Hopkins have a pipe line to Saudi Arabia, and we are importing human feces to do our tests. We also need a good technique for cleaning the cysts so that we can do these tests in a controlled manner.

Having taken care of those logistics, then we will want to evaluate a variety of disinfectants on *Giardia* that infect man. Of course, it was mentioned in the Symposium that there is a need to evaluate the coliform group and other organisms as indicators for *Giardia*. Perhaps the military might go back and evaluate water purification tablets for *Giardia* inactivation. Then, having taken care of the problem under the clean system, we can move into the dirty system and run similar tests in sewage and in raw and digested sludges. We may also raise the nagging questions of the possible health effects when wastewater and sludge reuses are comtemplated.

There are certainly other areas of concern, including medical management, that require better answers, but perhaps this will suffice for now.

G. ROBECK: Thank you, Dr. Kawata, for your perspective. We now have a few minutes for any further suggestions for research as seen from the point of view of anyone in the audience. Please feel free to express either your questions to the panelists or comments of your own about future needs in research.

S. ERLANDSEN: I have a question for Mr. Davies. It is very interesting to talk about the possiblity of infecting animals with *G. lamblia*. I wonder if you have carried that work one step further and shown from these same animals— and I guess the rat would be a very interesting model here since supposedly one cannot transmit *Giardia* from rats into humans—if you have those animals and they supposedly are carrying *G. lamblia*, can you take those cysts and put them into a primate and grow them?

R. DAVIES: I do not know about putting them back into primates, as we do not have any of those to work with. We are using SPF beagle puppies as a human surrogate, and we feel that anything that will infect humans will infect these dogs. We can take the rat *Giardia* and put it into beagle pups, but that is about as far as we have gone.

I believe that beaver are an extremely good reservoir and a continuing source of *Giardia* in watersheds, but I think we also should look at these as a good surveillance animal. In three different watersheds in Colorado I have gone up to a certain point and all of the beaver are infected near these beaver dams or ponds, but upstream they are clean. One point was Ashcroft, where I had reports of dog feces being washed into the river. Another place was on Beaver Creek, where all of the beaver were infected near two summer homes, but above that point none were infected. On Willow Creek, as long as the river was alongside campgrounds, beaver were positive for *Giardia*. Once there were no more campgrounds with heavy human use alongside that river, beaver were once again negative. On the Frazier River beaver were negative above the sewage plant and positive below the plant. Beaver may be a good sentinel animal for surveillance work.

G. ROBECK: Do you find *Giardia* in the effluents from the sewage discharges?

R. DAVIES: We have not tried to examine that. The beaver ponds were approximately 50 yd below the point where the effluent left the sewage plant.

S. ERLANDSEN: For those of us who are not fortunate enough to live close to beaver ponds but live in cities that have big storm drains and sewage areas, if we are going to consider the rat as a possible intermediate for cyst transmission, and rodents, muskrats and the like, how much contribution do they make to the cyst content of sewage versus the human source itself?

G. ROBECK: Do any of the panelists have any comment on this?

L. McCABE: We have a project in Washington state that is trying three techniques of surveillance of water supplies by filters, animal surveillance of each watershed, and through state laboratory reports. So the point of using animals as an indicator of how bad the watershed might be is being evaluated.

D. PRICE: In response to Dr. Kawata's comments, we are working with migrant communities in Florida. We are finding *Giardia* rates of up to 70% in migrant childcare centers, and in the general population it is running somewhere between 12 and 17% and in day care centers in Lakeland and Tampa, we are running about 8 or 10%. Overall, Florida is reporting a *Giardia* stool positivity rate of about 3.8% in the total population.

I have a comment for Mr. McCabe regarding the use of sewage. This is used in a number of places for golf courses and gardens, and one thing we are finding is that *Ascaris lumbricoides* and *Toxocara* from dogs are viable for years after this material has been passed unless it has been heated or is in a place where it gets enough sunlight to destroy the eggs. We have not seen *Giardia* at all in any of this material out in the field.

F. FROST: Our data on animal surveys would support Mr. Davies' findings. We have about 60 samples from protected watersheds, all of which were negative animal samples. We have about a 33% positive rate in muskrat, and we have beaver throughout the state with a 10% prevalency, so I think our data are in support of the suggestion of how people are acquiring it.

D. DEGIUSTI: I would like to know whether there are any ongoing programs on examination of the domestic rat for *Giardia*. There are resources available throughout the entire country, and I think that one of the problems is coordination. For example, in Detroit, we are doing a survey of *Leptospira* in rats. It never occurred to me to examine these same rats for *Giardia*. If there are ongoing programs, I think we ought to have some effort at coordination to utilize those resources.

G. ROBECK: Certainly we in EPA are always obligated to look to other agencies, local, state or federal, to develop the background information that we need to carry out our responsibilities and we are trying to do that from existing sources, but we want to be able to know how to use our limited resources to best advantage. Mr. McCable or Dr. Schultz, you might want to respond to that as you see it now or in the future.

M. SCHULTZ: I do not know of any studies presently on *Giardia* in rats. It would be interesting to study but then one would inevitably ask what does it mean.

R. OWEN: When I was at CDC we were sensitized to the problem of treating asymptomatic *Shigella* and *Salmonella* because of the potential for developing drug resistant organisms. I wonder if we treat asymptomatic *Giardia* infections if we are not going to come up with the same problem.

M. SCHULTZ: I do not think there has been any evidence for resistance developing against the anti-giardial drugs. Dr. Wolfe has had the most experience. There is resistance in malaria protozoa, but I cannot think of any for this.

M. WOLFE: Metronidazole has been used in amebiasis and for *Trichomonas* for many years, and I do not know of any evidence of resistance in people. Lower doses seem to be effective in *Trichomonas*. At least analogizing to other protozoan parasites, there is no evidence of resistance so far.

G. ROBECK: It would be fair to say that there was a lot of coordinating to do for this conference. I think it has been reasonably successful thanks to Walt Jakubowski and John Hoff, and to the various federal, state and local agencies, that have made their technical and scientific contributions. The Proceedings should serve as a good basis for the state of our knowledge and where we need to go with our regulatory actions as well as research.

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